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SOME REMARKS CONCERNING KUBISAGARI OR VERTIGE PARALYSANT.[1]

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An interesting disease has prevailed for a considerable period of time among the working peasant class of Japan, in the Northern Provinces, especially in the neighborhood of Homori and Iwateken. The principal symptoms consist in a dimness of vision, ptosis, diplopia; difficulty in speech, in deglutition and in mastication, as well as weakness in the muscles of the back of the neck and of the extremities. At a later time the investigations of my assistant, Takemura, demonstrated that this same disease occurs in some villages of Tokushimaken on the Island of Shikoku, but in a less pronounced form. My investigations in the regions mentioned above, undertaken in the years 1894 and 1895, demonstrated the affection to be identical with endemic paralytic vertigo observed by Gerlier in Ferney, Switzerland. So far as is known to me, no similar reports have come from other parts of the world and therefore I have called your attention to this disease.

Endemic paralytic vertigo (or kubisagari) has, if I can compare it with a disease known to you, a great similarity to myasthenia gravis, with this difference, that our disease appears more generally in the summer time in restricted neighborhoods where it is endemic and epidemic, and that the paresis appears simultaneously in various muscular regions. The symptoms are as follows:

1. Eye symptoms, namely, ptosis, dimness of vision, diplopia, hyperæmia of the papilla and its surroundings.
2. Motor disturbances of the tongue, and lips and interference with mastication, more seldom with deglutition.
3. Paresis of the muscles of the back of the neck and gluteal region.

[1] Read at the Fourth Annual Meeting of the Philippine Islands Medical Association, Manila.

4. Paresis of the muscles of the extremities.

According to Gerlier there may also be added neuralgic radiating pains to the back of the neck, back and to the periphery (such as forehead, shoulder and arms); more seldom an increase of the secretions of the nose, tear ducts and salivary glands is observed. Cramps, anæsthesia, hyperæsthesia, and disturbances of the organs of sense, or of the bladder and large intestine are always lacking.

To begin with, the symptoms observed in the eyes, obscureness of vision and ptosis, are the most important. The first of these begins with a blurring of the contours of objects which are observed; the patients state that they see everything as if it were surrounded by a fog. Some remark that they see near objects indistinctly, with others it is the reverse, still others have impaired vision both for near and far objects. In addition to the dimness of vision, ptosis is a frequent symptom and one which is more easily shown in an objective manner. This can exist in a slight degree even at times when the patient is otherwise apparently free from the disease. It appears in different degrees; it may be so slight that it only gives the patient a sleepy appearance or it may be so pronounced that only a hair-like slit is seen between the lids. It is brought about by a paresis of the levator muscles of the upper lid and is generally bilateral. Diplopia is less frequent and according to my observation is caused by a paresis of the rectus internus muscle. Gerlier, in addition, mentions photophobia, photopsia and disturbances in the color sense. Eperon, Sulzer and I demonstrated hyperæmia of the papilla, the optic nerves and their surroundings, during the attack.

Disturbances of speech, of the movements of mastication and deglutition are only seen in severe cases. During lengthy conversation the speech gradually becomes more and more indistinct and difficult to understand, because of increasing weakness of the tongue and lips. The muscles participating in mastication and deglutition at the same time lose their normal strength, so that the patient soon is unable strongly to bite on a finger thrust into his mouth, and finally he is unable even to swallow water. It is therefore not a rare occurrence that a person suffering from *kubisagari* loses all his food from his mouth during a meal, instead of swallowing it.

The paresis of the muscles of the back of the neck, back and extremities is observed most frequently when the peasants are working in swampy rice fields bent over their work, as occurs in planting rice and in weeding. Under these circumstances they first observe pressure and heaviness in the back of the neck, the head sinks forward and can only be raised with difficulty, so that in these cases a bandage is tied over the forehead and fastened by a string to the belt, in order to allow the sick one to work. The paresis of the muscles of the loins is manifested by the fact that the sufferer finds difficulty in raising himself to an erect posture after bending forward, and he must support himself by this hands on his thighs or hips;

he also complains of weakness in the body. Paresis of the extremities is manifested by dropping objects from the hands, or the patient moves painfully with a cane or along a wall or similar support. As the ptosis and dimness of vision is in these cases generally intense, the patients are usually compelled to keep quiet and await the passing away of the attack. All the motor disturbances first appear in muscles which are exerted, especially in cases of repeated, identical movements, as for example, during mowing with a scythe, pumping, marching, chewing, writing, etc.

The symptoms given above do not appear simultaneously nor with equal intensity in all cases, but one more generally finds them together in severe attacks; in the lighter ones there is either only ptosis with dimness of vision, or in addition only weakness of the muscles of the back of the neck.

The length of the attack is generally short, it rarely exceeds ten or fifteen minutes, but it reappears readily as soon as the customary work is continued.

The patients are well in the intervals between the paroxysms. They are neither anæmic nor nervous; the liver, spleen and other organs show no abnormalities, the blood contains no parasites either during the attacks or in the interval, the corpuscular elements are also not materially altered. Only patients who have suffered from repeated, severe attacks are left with a certain degree of ptosis and weakness of the muscles of the back of the neck, trembling of the hands, uncertainty of gait and speech (Gerlier) as well as increased reflexes.

Causative factors of the attacks are bodily exertion, notably labor in a stooping position, and repeated, uniform motions, especially on an empty stomach or after the ingestion of food difficult of digestion. Writing, reading, steady attention, mixing with crowds of persons and similar circumstances also act in a similar manner. On the other hand, the attacks are diminished or stopped by rest and change of location. This fact renders the study of the disease in hospitals almost impossible.

In regard to differential diagnosis, in the first place myasthenic paralysis must be taken into consideration as in this disease also, generally by repeated movements, the muscular strength gradually diminishes. On the other hand, in kubisagari there is sometimes a weak indication of the myasthenic reaction, but kubisagari is distinguished from the above-named disease by its endemic-epidemic character as well as by its sudden appearance, by the dimness of vision and by the rapid and more general extent of muscular weakness, etc. It is scarcely necessary here to enter upon a more exact discussion of the differential diagnosis between the disease and neurasthenia (ordinary paroxysmal lameness.)

The etiology of this peculiar disease is obscure. The superstitious peasants in Japan have thought of a supernatural influence of the wandering spirits of the dead who have found no resting place. The Swiss peasants believed in witchcraft. Ladame contends that

the epidemic spreading of the infection may be attributed to obscure psychic influences. David suggested adulterated alcoholic beverages, or a poisoning by bread or lentils. Other physicians in Japan and in Europe believe that latent malaria is the cause, but the clinical picture and the blood examination are against this view. Gerlier was the first who emphasized the close connection between this disease and those who work in horse's and cow's stables. After he had excluded the possible etiologic factors, one by one, he emphasized that it appears most frequently in peasants, day laborers, or persons who are occupied with the care of cows and horses, whereas landowners and women are free from endemic paralytic vertigo. Kubisagari also is general in those regions of Japan where a portion of the dwelling is used as a stable for horses or cows and when there is no idea of cleanliness. If we compare the region in Switzerland, where endemic paralytic vertigo is frequently encountered, with Aomori and Iwate, where kubisagari is endemic, we observe great differences in the geological structure of the region and the food of the peasants. Only one point is in common—in Switzerland the custom of sleeping in the stable and in Japan the imperfect separation of stable and dwelling.

Gerlier maintains "dans le bassin de Léman, il est d'usage qu'on couche á l'etable, ce qui n'est pas admis dans les cantons de Berne et de Tribourg ou la maladie est inconnue." With us in Japan the region of Aomori and Iwate is the territory where agriculture and the raising of cattle take place side by side, so that in each peasant's cottage, horses or cattle are maintained and cared for more carefully than the children. The inhabitants of this region generally have the barn so arranged that a part is used as a stable, and only an incomplete partition exists between the two spaces, so that not only the air but also insects have free access to all parts. The structure of the houses is planned for the winter months. The stable, however, is the place where throughout the year, processes of decomposition are going on and where there is a certain degree of heat in conjunction with imperfect lighting, a true incubator of micro-organisms, for the manure is only cleaned out twice a year.

At this point our knowledge ends. Is the etiologic factor an animal or a vegetable micro-parasite? Is the disease transferred by air, food, insects or domestic animals? The answer to these questions must be left to the future. Horses and cattle seem to resist this disease, but cats and chickens have been observed to be attacked by it, but much more rarely than human beings.

Prophylactic measures are the removal from the neighborhood of horse's or cow's stables and the avoidance of such places for the midday or night sleep. No great hope can be entertained from the use of medicaments, the best results are obtained from a combination of potassium iodide and arsenic. So far as is now known, no deaths have resulted from this disease, although it runs a course with bulbar appearances.

THE INVESTIGATIONS CARRIED ON BY THE BIOLOGICAL LABORATORY IN RELATION TO THE SUPPRESSION OF THE RECENT CHOLERA OUTBREAK IN MANILA.

By Richard P. Strong.

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The epidemic of Asiatic cholera which has recently passed through these Islands and has now subsided, occasions me at this time briefly to summarize the work of this laboratory in connection with the suppression of the outbreak. In all, there were 7,085 cases of cholera with 5,243 deaths reported by the Bureau of Health for the Philippine Islands. Dr. Victor G. Heiser,[1] Director of Health, in an admirable article, has recently discussed the origin and history of the outbreak and the general hygienic measures employed in combating the disease. My remarks will be limited to the laboratory measures carried on in connection with the epidemic.

The first case of cholera discovered in the outbreak occurred in Bilibid Prison on August 23, 1905. An autopsy was performed and a bacteriological diagnosis of cholera was reported sixteen hours later to the Bureau of Health. The Philippine Islands had supposedly been entirely free from cholera for the preceding seventeen months. Following the laboratory diagnosis of the first case, others suspicious of this disease were discovered by the representatives of the Bureau of Health, and within the next few weeks a positive bacteriologic diagnosis of cholera had been rendered by the laboratory in over one hundred instances. Soon after the report of the first case was made public, numerous specimens of the fæces of other patients suspected of having cholera were sent to the laboratory for bacteriological study and throughout the course of the epidemic examinations were carried on by members of the laboratory staff either in the central building or in the several hospitals where the suspected cases had been brought. An assistant of this laboratory was also stationed at the cholera hospital and was prepared at any hour of the day or night to undertake the bacteriological diagnosis of the cases admitted. The cholera spirillum was found present in 412 of 582 specimens of

[1] *Am. Med.* (1907), **48**, 856.

fæces examined, and in 304 autopsies performed by members of the laboratory staff on cases supposed to have died of cholera, the diagnosis of this disease was confirmed in 260; 129 specimens of drinking water, collected from reservoirs, wells and other sources of supply were also sent to be examined for infection with the cholera spirillum, but from only 3 of these was this organism isolated.

METHODS EMPLOYED IN THE BACTERIOLOGIC DIAGNOSIS.

Several methods were employed in performing the bacteriologic diagnosis from the fæces, an attempt being made in each instance to secure as prompt a result as possible. All methods which were not based upon the isolation of a pure culture of the cholera organism to be employed in the subsequent tests proved at times to be untrustworthy. The one which was demonstrated to be perfectly reliable in practically all acute cases and by means of which, in addition, a definite diagnosis could usually be reached within six to eight hours and almost invariably in from sixteen to eighteen hours, was as follows: Numerous alkaline agar plate cultures were prepared directly from the cholera stools, some being inoculated with large and others with small portions of the fæces, various dilutions being prepared; the cultures were placed at 37° C. and as soon as the colonies became sufficiently developed, those which resembled colonies of the cholera spirillum were suspended in saline solution. Agglutinative and bacteriolytic tests by the microscopic method were then performed with them and a standard *fresh* cholera serum in proper dilutions. The morphology and motility of the organism were also noted. Frequently, after twelve to sixteen hours from the time of the inoculation of the plates, sufficient growth was obtained in addition to carry out the Pfeiffer bacteriolytic test with the same cholera serum, in the abdominal cavity of a guinea pig. This method frequently, although not invariably, proved to be the quickest means by which a diagnosis could be made, and the preparation of the plate cultures from the suspected fæcal material soon became a routine one in the laboratory. In case a positive diagnosis was reached by methods requiring a briefer period of time, the subsequent agglutinative and bacteriolytic tests by the first described method were not always performed, but if a negative result was obtained by the briefer procedures, then these tests were carried out and they sometimes finally resulted in establishing a diagnosis of cholera.

Another method which frequently could be relied upon for diagnosis in case of a positive result, consisted in making the inoculations from the stool directly into tubes of 2 per cent peptone solution and of the performance of the agglutinative test by the microscopic method with drops of this medium taken from the surface and added to a cholera

immune serum. This test, together with the examination of the morphology and motility of the organism, was performed five or six hours after the inoculation of the cultures and in case a negative result was obtained, was repeated after from sixteen to twenty-four hours. Only when positive reactions are encountered by this method can the diagnosis be considered to be conclusive. In case of a negative result, a study of the plate cultures which should previously have been prepared should be resorted to. In some cases in which no agglutination of the organisms which has been cultivated in this way by the enriching process resulted, cholera spirilla were later isolated and identified by means of plate cultures. The success of the peptone solution method in securing a positive diagnosis obviously depends chiefly upon the number of cholera spirilla which exist in the stool.

A number of experiments in diagnosis were also carried on according to the method advised by Dunbar [2] and by means of which an immediate diagnosis of cholera may occasionally be made from the fæces. However, it is worth while to undertake this method only with specimens of excreta in which numerous organisms with more or less typical morphology of the cholera spirillum are present. In stools of this nature the reaction should always be attempted because of the immediate results which may sometimes be obtained. Care must be taken to distinguish pseudo-reactions, and only those cases should be considered as conclusive in which the agglutination is distinct and well marked. The reaction frequently failed in instances of undoubted cholera from which pure cultures of the cholera spirillum were later isolated and identified. The so-called cholera-red reaction, performed with peptone cultures and with nitrite free sulphuric acid, could only be considered in determining the diagnosis when a positive result was obtained, and even then the reaction could only be regarded as confirmatory from a bacteriologic standpoint. A single negative reaction, even though a satisfactory peptone media had been employed, could not be looked upon as an important argument against a positive diagnosis of the cholera organism, since different strains were found to vary in this respect. Obviously, the cholera-red reaction, unless performed with pure cultures of the spirillum, is entirely untrustworthy and the results can not be depended upon even as an aid in diagnosis.

No experiments were performed with the specimens sent for diagnosis, with the object of differentiating by means of the blood-agar of Prausnitz [3] the cholera spirillum from other cholera-like vibrous in the stools, the agglutinative and bacteriolytic tests having by practical experience proved to be satisfactory for clinical diagnostic purposes.

[2] *Berl. Klin. Wchnsch.* (1905), 42, 1237.
[3] *Berl. Klin. Wchnsch.* (1905), 42, 561.

notwithstanding the experiments of Kraus. The bacteriologic diagnosis in cases of cholera in the late stages of the disease was sometimes very difficult, and a careful study of the fæces by plate cultures, prepared both directly from the stool and from the surface of peptone solution cultures previously inoculated with large amounts (several cubic centimeters) of the stool, is sometimes necessary in such cases before the organism is isolated. Frequently, an examination of the agglutinative and bactericidal reaction of the blood serum of the patient in these subacute cases will render further assistance in reaching a diagnosis, particularly if the reactions are positive.

PREPARATION AND FURNISHING OF CHOLERA IMMUNE SERA FOR DIAGNOSIS.

In addition to the preparation of fresh cholera immune sera for diagnostic purposes in the central laboratory, such sera were also prepared and furnished to various physicians and institutions in the city and in certain of the provinces for use in the bacteriologic diagnosis of the disease. No dried cholera serum was issued, it having been found practicable and more desirable to furnish a fresh serum by means of which not only an agglutinative test, but also a bacteriolytic one under the microscope according to the method of Bordet could be performed. It was found that a single intravenous inoculation of a rabbit with the immunizing substances extracted from about 60 to 70 milligrams of a virulent cholera organism would furnish a serum of sufficient value for all practical purposes in diagnosis. The animal may be bled to death on the sixth or seventh day after such an inoculation and the serum separated. Such sera almost invariably show an agglutinative value of from 1:1,000 to 1:2,000; values as low as 1:800 have been obtained only in exceptional cases. The bactericidal almost invariably exceeds the agglutinative value of the serum prepared in this manner, the different sera reacting bactericidally in amounts of from 0.2 to 0.05 milligram.

After the serum had been standardized it was sealed in test tubes and was ready for delivery. Fresh serum produced by this method was kept on hand throughout the epidemic.

PROPHYLACTIC INOCULATIONS.

The laboratory in addition to the diagnostic work was particularly occupied in the preparation and standardization of cholera prophylactic and in the performance of the protective inoculations against the disease in certain badly infected cholera districts in Manila and the provinces.

The prophylactic prepared and employed consisted of the immunizing substances extracted from the cholera spirillum and suspended in

saline solution.[4] The method of its preparation has been described in detail in former articles from this laboratory. It will be sufficient to state here that an organism known to possess high immunizing and peptonizing powers and one of maximum virulence is selected and grown upon the surface of large test tubes containing 1 per cent alkaline agar. After twenty hours, the growth of one-half of the number of inoculated culture tubes is suspended in 0.85 per cent saline solution, 1 cubic centimeter being employed for approximately every 30 to 35 milligrams of bacteria. The suspension is heated for one hour at 60° C. and then placed at a temperature of 37° for from three to four days. At the end of this time its sterility is tested, it is filtered through a Berkefeld candle and the filtrate saved. The remaining half of the twenty-hour agar cultures is suspended in sterile distilled water, 1 cubic centimeter to each 30 to 35 milligrams. This suspension of the *living* organisms is then placed on an electrical shaking machine and throughly shaken for from three to four days. At the end of this time cultures are taken to ascertain if the growth is pure and the suspension is then also filtered through a Berkefeld candle. The two filtrates are subsequently mixed in equal proportions and carbolic acid added to 0.5 per cent. The prophylactic is finally bottled in glass flasks, the smaller ones being sealed in the flame. Two cubic centimeters of this mixture represents an adult dose. After the sterility of each sample of the vaccine has been tested by animal inoculation and by anaërobic cultures, it is standardized according to the number of units of immunity to which it gives rise after inoculation; one unit of immunity equaling the amount of immune serum which will protect a guinea pig of 250 grams weight against the intraperitoneal inoculation of ten times the fatal dose of living cholera organisms. If 1 cubic centimeter of the vaccine, when injected intravenously into a rabbit, does not give rise to at least 10,000 units of

[4] The process by means of which this prophylactic is prepared may be regarded as the outcome of the experimental work performed by Koch, Neisser and Shiga, Wassermann, Brieger, and myself. The idea of using the immunizing substances (free receptors) extracted from *Bacillus typhosus* and *Bacillus dysenteriae* for producing immune sera originated in Ehrlich's laboratory with Neisser and Shiga[5] in 1903, and that of using the extracted free receptors of the cholera spirillum for prophylaxis against cholera in Wassermann's laboratory a few months later (during the same year) where I[6] was able to carry on the first extensive and conclusive experiments in regard to its value. Later Shiga[7] advocated and used the method for preparing a prophylactic against typhoid fever.

Brieger and Mayer[8] called attention to the advantage of extracting the soluble substances of the organisms by shaking in distilled water.

[5] *Deutsche med. Wchnsch.* (1903), **4**, 61.

[6] *Am. Med.* (1903), **6**, 272.

[7] *Berl. klin. Wchnsch.* (1904), **4**, 79.

[8] *Deutsche Med. Wchnsch.* (1904), **30**, 980.

immunity, it is not issued for human inoculation. No method of standardizing the small amount of anti-endotoxin which the prophylactic will give rise to in animals has been found to be either practicable or of value. During the period of the epidemic the prophylactic was manufactured and used in large quantities and a sufficient supply was kept on hand for emergencies.

EFFICIENCY OF THE PROPHYLACTIC.

I called attention to the fact in my previous publications on the subject of protective inoculation against Asiatic cholera, that animals could invariably be protected against a subsequent cholera infection with even multiple lethal doses of the organism as the result of a single inoculation of this prophylactic, in doses of from 1 to 5 cubic centimeters: and that in addition agglutinative and bactericidal substances became developed in considerable quantities in the blood sera of such animals. However, the antitoxic value of these sera was only moderate. It was also demonstrated that the antibodies which developed in the blood of individuals after inoculation with the prophylactic were identical with those which were encountered in patients convalescent from cholera, and, in addition, that these immunizing substances were frequently present in greater amounts after vaccination than after a natural attack of the disease. Moreover, it was shown that animals which contained these same antibodies in sufficient quantities in their sera were also invariably immune to cholera infection, the amount of the immune bodies in the serum being proportional to the degree of immunity to infection possessed by the animal.

From a single intravenous inoculation of rabbits with an amount of the prophylactic containing the receptors extracted from 2 oesen of a virulent cholera organism, sera were obtained which showed an agglutinative value of from 1:300 to 1:600, and a bactericidal one of from 0.1 to 0.08 milligram, while from a similar inoculation of the receptors extracted from 12 oesen of the same strain, sera resulted showing agglutinative values of from 1:600 to 1:1,000 and bactericidal values of 0.08 to 0.04 milligram. From a single intravenous inoculation of $\frac{1}{2}$ oese of a *living* agar culture of this same cholera strain, sera were obtained with an agglunative value from 1:400 to 1:800 and a bactericidal one of from about 0.1 to 0.06 milligram.

It is important to observe that 0.5 oese of the living organism gave rise to sera of almost the same value as did the receptors which could be extracted from 2 oesen of the bacteria, but that the receptors extracted from 12 oesen of the organism furnished sera of far greater value. The best antitoxic serum which was produced following a single intravenous inoculation of the prophylactic was found not to be able to neutralize above four lethal doses of the cholera endotoxin.

From the subcutaneous inoculation of rabbits with 5 cubic centimeters of this prophylactic containing the receptors which could be extracted from 40 oesen of the same strain, sera were obtained with an agglutinative value of from 1:500 to 1:600 and a bactericidal one in from 0.14 to 0.1 of a milligram. Animals have been found to retain these immune bodies in their sera for as long a period as one year.

A brown powder was obtained by evaporating the prophylactic to dryness in a vacuum. This was placed in sealed tubes and when desired for use redissolved in saline solution. The intravenous inoculation of rabbits with from 3 to 10 milligrams of this redissolved powder furnished sera of an agglunative value of from 1:50 to 1:100 and a bactericidal one of from 2.5 to 0.25 milligram. These sera were obviously of much lower value than were those which resulted from the inoculation of the prophylactic before evaporation.

In man after the subcutaneous injection of 2 cubic centimeters of the prophylactic, sera showing agglutinative values of from 1:40 to 1:600 and bactericidal ones of from 1 to 0.25 milligram were obtained.

The advantages which this prophylactic seems to possess over other forms of anti-cholera inoculation are:

First, there is practically no local reaction or only a slight one after its use, the irritating oxidizing substances which existed in the bodies of the bacteria and which have nothing to do with the immunizing substances, having been removed.

Second, we therefore are able to inject an amount of these immunizing substances which is from fifteen to thirty times as great as would be practicable if either the living or killed bacteria were inoculated. Both Haffkine and Murata in their extensive inoculations in human beings injected from 2 to 4 milligrams of culture. In our human inoculations the immunizing substances extracted from about 60 to 70 milligrams of culture are inoculated, hence a higher immunity is obtained by this procedure.

Third, the prophylactic may be sealed in flasks and stored ready for use and it preserves its immunizing properties for at least a year.

The great disadvantage which the method possesses is that each step of the manufacture of the prophylactic must be carried on with great care. It is obvious that the product which we recommend and employ is far more difficult to prepare than is either a simple suspension of the killed or of the living cholera organism. A well-equipped laboratory and trained assistants are necessary for its manufacture. However, it is equally clear that a higher immunity against cholera infection can be obtained by a single injection of this prophylactic than by single inoculations of either the killed or living organisms. The reasons for this have already been emphasized.

Therefore, it has been shown that it is easy to produce in man a

high bactericidal serum by the injection of the prophylactic just described. However, the question arises whether such a serum in man really represents an immunity against the disease Asiatic cholera—that is, does it protect the individual against intestinal infection? This question apparently resolves itself chiefly into one as to whether the organisms which give rise to the symptoms of the disease actually come into contact with the fluids of the body.

All articles upon the pathologic histology of cholera agree that the spirilla are found in the superficial layers of epithelial cells of the mucosa, and, sometimes in large numbers, penetrate well into the submucous lining. Exfoliation of the epithelium is almost a constant occurrence in severe infections, and in cases of longer standing the organisms are sometimes encountered in large numbers at the bases of erosions or ulcerations which have resulted in the large intestine. In addition, the mucosa is usually distinctly œdematous, and there obviously is no doubt but that the bacteria in any of these situations would come into contact with whatever immunizing substances might exist in the blood serum, and if bactericidal substances were present in sufficient strength, the bacteria would be destroyed. However, we know that very large numbers of the cholera spirilla remain in the lumen of the intestine in the rice-water discharges and do not invade the intestinal coats. We do not know whether these organisms which so remain in the bowel give rise to the symptoms of the disease, since satisfactory evidence that the cholera spirillum produces a soluble toxin has not yet been presented; but even granting for the moment that they do, then what influence if any would bactericidal substances in the blood serum exert upon them? We are aware that one of the most common and striking findings at post-mortem examinations in Asiatic cholera is the so-called "rice-water" contents of the small intestine. The characteristics of the rice-water stool are due to the flakes of mucus and the epithelial cells which appear in suspension in the liquid contents. If we examine these floccules of mucus bacteriologically we find that the spirilla are usually most abundant in them, sometimes in almost pure culture. However, this mucus is largely a secretion of the epithelial cells of the intestine, although it is usually also mixed with a certain amount of serum and such a secretion will probably possess the same bactericidal substances as the blood serum itself, just as the sweat, the tears, and the milk of immunized individuals contain these antibodies. Indeed, it seems very probable that the intestinal epithelial cells which give rise to the mucus are perhaps particularly able to produce these immunizing substances, since there is probably a special affinity or combining power between these cells and the cholera organism, which is demonstrated by the production of toxic substances in far greater amount in

the human intestine than the injection of these bacteria into the circulation, or beneath the skin either of man or animals ever gives rise to.

Having investigated the agglutinating power of a fresh extract obtained from the intestinal epithelial cells of two rabbits previously inoculated intravenously with the immunizing substances extracted from the cholera organism by autolysis, and having found traces of agglutinins present in these extracts, it occurred to me that it would be advisable to undertake the investigation of the bactericidal value of the rice-water stools obtained from cholera cases and also to examine whether the cells of the intestinal mucosa in rabbits immunized with our cholera prophylactic possessed an increased affinity for cholera receptors. It was also of interest to discover if extracts of these cells from the inoculated rabbits possessed bactericidal properties. This study was undertaken by Dr. Edwards of this laboratory who reported his results at the Third Annual Meeting of the Philippine Islands Medical Association in 1906. Owing to the difficulties encountered in the technique, produced by decomposition of the extracts obtained from the intestinal cells, admixture with other bacteria, etc., he was not able to arrive at any satisfactory conclusions in regard to these questions.

It has been supposed that when cholera organisms are injected intravenously into animals, the immunizing substances are anchored particularly to the cells of the spleen, the bone marrow, and lymph glands, since it was in these organs, according to the investigations of Pfeiffer and Marx,[9] that the specific protective substances seemed particularly to be formed. However, Wassermann and Citron[10] demonstrated that the location of the development of the immunizing substances depends to a large extent upon the point at which the injections of the corresponding bacterial antigens were made. Nevertheless, the combining power of intestinal epithelial cells for the receptors of the cholera spirillum has not been definitely determined,[11] although as has been mentioned it seems not unlikely that in the human intestinal infection the epithelial cells of the mucosa may possess receptors with special combining powers for the corresponding cholera antigens and hence

[9] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1898), **27**, 272.

[10] *Ibid.* (1905), **53**, 331.

[11] Brieger and his assistant took *per os* repeatedly from 5 to 15 centimeters of Brieger's vaccine (aqueous extract of typhoid bacilli) but no development of bacteriolysins occurred in their blood.* This experiment throws no light upon the subject since perhaps, the bacterial antigens were changed or destroyed by the gastric and intestinal juices before their immunizing power was exerted upon the intestinal cells.

* Bischoff, H.: Das Typhus-Immunisierungsverfahren nach Brieger. *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1906), **54**, 298.

give rise to the production of amboceptors (immunizing substances) in excess, which may pass off in the exudates from the cells, as for example in the intestinal secretion containing mucus and serum. However, while it is interesting to review this evidence that the organisms in the intestine in Asiatic cholera do, indeed, come into contact with the serum and tissues of the host, the most definite proof that this really takes place is furnished by the fact that after an attack of the disease, both bactericidal and agglutinative substances are produced and may be demonstrated in the blood serum of the individual. It therefore seems to be proved that if cholera organisms should find their way into the intestine of one whose blood serum possesses a high bactericidal power against the spirillum, the individual in question would be in a much more favorable condition for overcoming the infection, or indeed of throwing it off entirely, than if no such bactericidal substances were present.

Haffkine's extensive statistics demonstrate this fact conclusively and show the value of protective inoculation against this disease, the number of cases of cholera among the inoculated being only about one-tenth that observed in the uninoculated. I have no such extensive statistics to offer in regard to the use of our prophylactic, since we have pursued our inoculations during the past year only in those districts where it was thought that the value of the inoculations might be clearly determined. The first town in which extensive vaccinations were practiced was Angat and its barrios in the Province of Bulacan. The village is situated directly upon a small river from which it derives its entire drinking water supply. Considerable drainage from the town in wet weather passes into this river. Cholera was not present in the town at the time the inoculations were performed, but it had been present there a short time before and it was thought probable that it might recur in that locality during the rainy season. About one-sixth of the population of the village—that is, all who volunteered, 1,078 in number—was injected with the prophylactic. A few months later cholera appeared in the village and 122 persons were stricken with the disease, 121 of whom were among the noninoculated. In the villages of Siniloan, Mabitac, and Malolos, 2,706 persons were inoculated, but since the inoculations were performed there has not been sufficient cholera in these localities to draw conclusions of any particular value as to their efficacy. However, only three of the entire number of persons inoculated have contracted the disease. In Bilibid Prison a little over one-half (1,838) of all the inmates was inoculated. During the twelve months following the injections, according to official reports of the Bureau of Health, there have been twenty-four cases of cholera in the prison, only four of which were among the inoculated.

REVIEW OF THE RECENT WORK UPON PROTECTIVE INOCULATIONS AGAINST CHOLERA.

Having briefly outlined the work carried on in this laboratory in connection with prophylaxis against cholera, I wish to review the results which several other observers have obtained with different methods of anti-cholera inoculation, since the publication of my last article upon the subject.[12]

Bertarelli[13] in April, 1905, performed a few experiments in cholera immunization, using for the injections the receptors of the spirillum separated by autolysis, but the strength of the autolytic product employed is not given. He inoculated himself subcutaneously with 3.6 cubic centimeters and a rabbit with 5 cubic centimeters of such a prophylactic and was able for more than six months after the inoculation to demonstrate agglutinative and bactericidal substances in the blood in each instance. During the previous year, I had already shown that in the blood these antibodies persisted for a longer period of time than six months after inoculation with the immunizing substances extracted by autolysis from the organism. Bertarelli's experiments do not appear to be sufficiently extensive to throw much light upon the value of the method of immunization with the autolytic extracts of the cholera organism, although he apparently considers this method of inoculation of value.

Heller,[14] (Schweizer Serum und Impfinstitut, Bern), after pointing out the advantages of protective inoculation against cholera with cholera nucleo-proteid prepared by Lustig's method, reports the results of an experiment in a rabbit in which the animal had finally received 0.25 gram of the nucleo-proteid. The serum was then tested and showed an agglutinative value in a dilution of 1: 400. By finally inoculating 0.8 of a gram of the nucleo-proteid this value was increased to 1: 1,000. A rabbit which was inoculated with the entire cholera organism produced an agglutinative serum in a dilution of from 1: 1,000 to 1: 3,000. The author emphasizes the fact that the injection of the nucleo-proteid causes but a moderate reaction and gives rise to a high immunity which lasts for months, and that the prophylactic does not readily deteriorate.

Friedberger and Moreschi,[15] in a study of the comparative value of the active immunization of rabbits against cholera and typhoid infection obtained by different methods, performed numerous experiments with cultures of the cholera organism killed by diverse means and with others dried at high temperatures or autolytically digested. Their conclusions in this article throw but little light upon the value of autolytic digestion as a practicable means of obtaining the immunizing substances from the spirillum for use in cholera prophylaxis. In their conclusions regarding this subject, they state that with the Pfeiffer-Kolle method, or with one recommended by Loeffler (in which the bacteria are killed at 120° C.), autolysis carried on at body temperature produces no distinct influence upon the activity of the antigens in immunization, and that certainly these substances do not become increased. In another portion of their article they

[12] *Publications of the Bureau of Government Laboratories*, Manila (1904), **16**, 1, and *J. Infect. Dis.* (1905), **2**, 107.

[13] *Centrbl. f. Bakteriol. Orig.* (1905), **38**, 584.

[14] *Ibid*, **39**, 106.

[15] *Ibid*, 453.

point out that three days' autolysis of cholera cultures killed at 60° causes no loss of their ability to produce bacteriolytic substances upon injection.

Obviously, a given cholera organism is endowed with a certain number of receptors. It is difficult to conceive how these receptors could be increased by autolytic digestion of the organism. The advantage of autolytic digestion in obtaining the immunizing substances from the cholera spirillum for use in prophylaxis is that it permits these substances to be separated from other injurious and nonimmunizing substances in the protoplasm of the cell, and hence permits of the inoculation of a larger dose of immune bodies than does the method in which the entire bacterial cell is injected.

A consideration of great practical importance—namely, the influence of the size of the dose upon the antibody production—is discussed by Friedberger and Moreschi, who point out that 1/500 oese injected intravenously gives rise to the production of the same amount of antibodies not only as 1/100 or even 1/10 oese, but to the same amount as does a 2,000 times larger injection, namely 4 oesen. These results of Friedberger and Moreschi have not been confirmed by other authors, and the immunity obtained in cholera must still be considered within certain limits to be proportional to the dose inoculated, as I pointed out several years ago.[16]

Schmitz,[17] in a very exhaustive article from the Institute for the Investigation of Infectious Diseases in Bern, calls attention to the immunizing value of cholera prophylactic prepared according to the method of Lustig, and shows that by its use animals may be immunized against cholera infection and that, following inoculations with it, both agglutinative and bacteriolytic substances develop in their sera. However, these antibodies according to his experiments were not produced in very great amounts, an agglutinative value of only 1:400 being obtained after an injection of 0.25 milligram of the vaccine and only one 1:800 after the size of the dose had been increased to 0.8 milligram.

B. Klein[18] performed a few experiments for the purpose of comparing the value of immunization with killed agar and bouillon cultures of the cholera organism with that produced by the autolytic extracts of the spirillum. In all, eleven animals were immunized, four with the autolytic extracts and five with the killed cultures. All were found later to be immune to cholera infection. In concluding his remarks the author quotes Wysokowicz, who states that it is still unproved how long the immunity is retained after inoculation with the autolytic extracts of the cholera organism and that the method of preparation of the prophylactic is more complicated than with that recommended by Kolle. However, in very susceptible persons the autolytic extracts are recommended because of the fact that the local and general reaction following their use is milder than when Kolle's method is employed.

Serkowski[19] during the epidemic at Lodz inoculated eighteen persons, eleven with killed agar cultures of the cholera organism and seven with the separated free receptors. Upon the later examination of the bactericidal properties of the blood serum of the inoculated, he found no difference in value between those vaccinated with the killed cultures and those with the extracts of the organism. However, he points out that the preparation of the vaccine according to the former method is much simpler. The size of the dose employed in either method

[16] *Publications Bureau of Government Laboratories, Biological Laboratory* (1904), **21**, 1. *J. Exp. Med.* (1905), **7**, 229.

[17] *Ztschr. f. Hyg. u. Infektionskrankh.* (1905), **52**, 1. *Centrbl. f. Bakteriol. Orig.* (1906), **41**, 118.

[18] *Centrbl. f. Bakteriol. Orig.* (1906), **41**, 118.

[19] *Ibid*, 255.

is not given. In the further inoculation of a number of human beings with killed cultures, in some of whom the injection was repeated a second and third time, it was demonstrated that there was a distinct relationship between the bactericidal immunity obtained and the size of the dose. However, there appeared to be no direct relation between the size of the dose and the agglutinative value of the blood, nor between the agglutinative value and the bactericidal power; neither did the number of vaccinations seem to be directly related to the formation of the agglutinins.

Meinicke, Jaffé and Flemming[20] have carefully considered the binding power of the cholera vibrio in relation to the production of immunity. Their experiments performed upon the relation between binding power and virulence are of great practical importance in regard to the subject of protective inoculation in man. They conclude that binding power and virulence are independent of each other, since in some cases the avirulent cholera organism revealed a greater binding power and in others a lesser than certain more virulent ones. They believe that the apparent quantitative differences *in the binding power between different cholera strains* can be explained by the qualificative differences in the structure of the receptors of the organism. They also conclude, although their experiments in relation to this point are few in number, that the virulence of a cholera culture bears no relation to its immunizing power. They were unable to confirm the work of Friedberger and Moreschi in regard to obtaining sera of as high a value from the intravenous inoculation of 1/100 oese of a cholera culture as from a much larger dose. Even with the intravenous inoculation of 1/10 oese they were able to produce sera of moderate value only in about half of the animals inoculated. Differences in the value of the sera were much greater when the small doses were used than when larger ones were employed. They believe that Friedberger and Moreschi's results can be explained by the fact that in immunization with such small doses, the value of the serum obtained depends largely upon individual variations in the animals furnishing the serum.

Fichera's,[21] experiments in relation to binding power and virulence are mainly confirmatory of those of Meinicke, Jaffé and Flemming. This author found that strains of the cholera organism which had been isolated for long periods of time still possessed the same binding power for cholera amboceptors as freshly isolated cholera cultures. Fichera also investigated the relation between the immunity produced and the size of the dose. He found, contrary to Friedberger and Moreschi, that rabbits inoculated intravenously with 1/100 of a 24-hour culture killed at 60° C. furnished sera which had an agglutinative value of about 1/10 or even less of that furnished by animals inoculated with 1/20 of the culture. The bactericidal value of the sera obtained from the inoculation of 1/100 of a culture was about one-fifth the value of the latter. The results of Friedberger and Moreschi, as the author points out, may be explained on the ground of individual variation in the immunity of the different animals. A human being was inoculated intravenously with 1/100 oese of a cholera culture killed at 60°, but no practical increase in the immune bodies of the serum was demonstrated, therefore the author does not recommend this small dose for active immunization. Fichera recalls that, with those methods of cholera immunization in which specific sera are added to the bacteria before inoculation, the immunizing value of the organism is lost in proportion to the saturation of its receptors with amboceptors before the injection. In case the vibrios were saturated, so to speak, with the serum, the animals were only immunized slightly or not at all.

[20] *Ztschr. f. Hyg. u. Infektionskrankh.*, Leipz. (1906), **52**, 416.
[21] *Centrbl. f. Bakteriol. Orig.* (1906), **41**, 576, 671.

Karwacki[22] inoculated nine persons with about 1 oese of killed cholera organisms suspended in saline solution. After five days a second dose of 2 oesen was injected, following which, apparently quite marked local reaction occurred. The reactions were usually milder after the second than after the first injection. After the first vaccination the bactericidal value of the serum was 0.02 in eight of the cases (50 units); after the second the sera showed values of from 2,000 to 10,000 units. The agglutination after the first inoculation in no case reached over 1: 50. In some instances there was no reaction in dilutions of 1: 5, while after the second vaccination the agglutination was in no case below 1: 5 and in one it had reached 1: 400.

Blell[23] (from the Institute for the Investigation of Infectious Diseases in Bern) has also reported in detail upon the value of cholera immunization with cholera nucleo-proteid. The agglutinative and bactericidal value of the blood sera of a large number of animals was studied. Many of these had received repeated and increasing doses of the prophylactic. In two cases the bactericidal value of the rabbit's blood was determined after single injections of 0.1 gram and 0.05 gram, respectively, of the nucleo-proteid and was found to be 5 milligrams and 10 milligrams. In the rabbits which had received a number of repeated inoculations, sera to a maximum value of 0.8 of a milligram were obtained.

The author also reports experiments from the results of which he believes that cholera immune serum produced by inoculation of the nucleo-proteid may exert a curative effect on animals which are inoculated with it in from one to four hours after infection with living cholera spirilla.

Finally, we have the report of Haffkine[24] which gives a summary of the work performed on anti-cholera inoculation in India. Haffkine refers to the recent work of Pfeiffer and Friedberger and of myself, which seemed to demonstrate that the vaccinating power of a cholera culture varies in direct relation with the degree of its virulence, a principle he remarks which served Pasteur for twenty years as a basis for his "*traitement intensif*" in rabies. Haffkine points out that for this reason in the beginning of his work on protective inoculation against cholera he sought to obtain a "*virus fixe*" with the cholera vibrio.

In his very extensive inoculations in man he has observed that the intensity and duration of the symptoms provoked by the subcutaneous inoculation of the living vibrios are directly proportional to the virulence of the culture and the quantity injected. He again describes the methods by means of which the fixed and the attenuated virus used in making the inoculations are prepared. He has found that if the cultures of the organism are killed by heat or by antiseptics such as carbolic or inorganic acids, or by other means, they retain the power of producing an immunity upon inoculation, but this is considerably reduced. The reaction produced after injection of the killed cultures was of the same nature as that brought about by the living ones, only it was less intense. Up to the year 1895 Haffkine always employed two vaccines, the first consisting of a culture attenuated by growing it in contact with the oxygen of the air, and the second, a virus of fixed virulence obtained by passage through guinea pigs. Since 1895, owing to the fact that it was found impracticable sometimes to give the second inoculation, experiments were made by Haffkine and Powell to see if the first vaccination with the attenuated culture could not be omitted. As much as $\frac{1}{6}$ of a gelatin culture of the fixed virus alone was injected in a large number of

[22] *Ztschr. f. Hyg. u. Infektionskrankh.*, Leipz. (1906), **54**, 39.
[23] *Ztschr. f. Hyg. u. Infektionskrankh.*, Leipz. (1906), **55**, 187.
[24] *Bull. Inst. Pasteur* (1906), **4**, 694 and 737.

cases without any serious illness resulting. Between April, 1896 and 1899, somewhat more than 6,500 cases were vaccinated in this manner. Powell, in a report of these cases in 1899, showed that among 6,549 nonvaccinated individuals there were 198 cases and 124 deaths from cholera, while among 5,778 of the vaccinated there were only 27 cases and 14 deaths.

Haffkine points out that while the vaccinated individual is obviously less apt to contract cholera than the nonvaccinated, if the former should actually be striken with the disease he is as likely to succumb to it as the latter (since no marked antitoxic immunity has been produced by the vaccination). The immunity following the vaccination may persist for fourteen months, after which time it diminishes and probably disappears. During the period of active immunity the number of cases of cholera among the vaccinated is but one-tenth of that observed in the uninoculated.

The statistics which Haffkine quotes in his paper conclusively prove the value of protective inoculation against this disease.

We see from this review of the recent work on protective inoculation against cholera that three observers, all from Bern, have reported upon the value of cholera nucleo-proteid as a means of immunization against the disease and have performed numerous experiments showing that antibodies enter into the blood sera of animals inoculated with this form of prophylactic. From single inoculations in rabbits, sera having as high an agglutinative value as 1:500 and a bactericidal one of 5 milligrams were obtained, but none were higher. However, these values are somewhat low when compared with those I have encountered in rabbits after a single inoculation of the prophylactic I have described.

Turning now to the experiments which have been performed by other observers, it may be seen that no very extensive studies other than my own have been made with the immunizing substances of the cholera spirillum obtained and separated by autolysis, either in man or animals. All of those who have reported upon the use of the method have apparently lost sight of what seems to me to be its most important advantage, namely, that when the immunizing substances are extracted from the cholera organism they may be injected in much larger amounts at one time than if the whole organism is used. I have inoculated myself with three oesen (6 milligrams) of a living, virulent, cholera organism at a single dose, and the local and general reaction experienced was such as to make me conclude that a larger amount than this would not be practicable nor desirable as a method for general inoculation. On the other hand, as I have pointed out, our routine method of human inoculation consists of the injection of the immunizing substances in colloidal suspension or in solution, extracted from 60 to 70 milligrams of the bacteria. Obviously, we are not able to separate and obtain all the receptors of the organism by the method I have employed. This was demostrated by my earlier experiments, when it was shown that $\frac{1}{2}$ oese of the living organisms furnished as good immune sera as the receptors extracted from 2 oesen of the same culture. However, the receptors

extracted from 12 oesen gave rise to far better sera. A much larger dose than ½ oese of the living organisms could not have been resisted by the animals, as they would have succumbed to an infection with the spirillum.

LOCAL REACTION FOLLOWING INOCULATION.

Several of the observers who have used in man the method of injecting extracts obtained by autolysis remark upon the fact that the local reaction is less marked than in the case of the inoculation of either the killed or living spirilla. There can be no doubt of this fact and we have had opportunity to compare the reaction produced by each of these methods in man as well as in animals. In animals the differences are very striking and may easily be observed. If a guinea pig is inoculated in the abdominal cavity and tissues overlying the abdominal wall with ½ oese of a virulent cholera organism of which the lethal dose is about 1/10 of an oese, or with 5 or 6 oesen of the same killed organism, at autopsy in the neighborhood of the track of the syringe needle there is found a hæmorrhagic and infiltrated area which is usually sharply circumscribed and of a bright or dark red color. If, on the other hand, a guinea pig is inoculated with a large dose—for example, 5 cubic centimeters—of the extracted prophylactic I have described, the animal may succumb to the injection from intoxication, but at autopsy no hæmorrhagic area will be observed about the point of inoculation. The walls of the abdomen are swollen and œdematous, but there are no evidences of an acute, inflammatory process such as occurs when the living or killed organism itself is inoculated. On the other hand, if inoculations in amounts just below the lethal dose are made with each form of prophylactic, no difference in the quality of the immunity can be detected in the two animals, variation only being found in relation to the *quantity* of the amboceptors; the animals inoculated with a large amount of the prophylactic yielding a better serum than those which had been given the living organisms.

Therefore, it seems unquestionable that there are other irritating substances in the cell of the cholera organism which have nothing to do with the production of the immunity, and it is these substances which may be separated to a large extent from the immunizing antigens by autolysis.

The action of these irritating substances in the cholera cell seems to be particularly active when a large amount of the organism in concentrated suspension is introduced into the tissues; thus, in deaths from Asiatic cholera we do not see markedly hæmorrhagic areas in the intestinal wall where the organisms are spread out over the surface of the mucosa, but if several oesen of the living organisms are introduced into the subcutaneous tissues in man, the hæmorrhagic condition may be produced, as I have demonstrated by an experiment performed upon

myself. Inoculation of the human being with the extracted prophylactic which I have described does not produce this hæmorrhagic change in the tissues. Haffkine in relation to this question and of the inoculation of this fixed virus says:

> En inoculation souscutanée au cobaye, le virus ne tuait pas, mais produsait une mortification des tissus et une eschare de grand extension.

If this local reaction is entirely due to the immunizing substances of the cholera organism, why are not similar lesions encountered elsewhere in the animal body? Cholera is an intoxication and it seems almost unreasonable to suppose that all of the toxin is bound locally, either in the intestine in natural infection or in the subcutaneous tissues after artificial subcutaneous injection; the immunizing substances are soluble and must pass to the other organs of the body. It rather appears that with the cholera spirillum, as with the diphtheria bacillus, we have to do also with substances within the bacterial cell which give rise to a local inflammatory reaction, but which have nothing to do with the true immunity in the disease. However, in cholera, of course the toxin is of an entirely different nature from that encountered in diphtheria and probably only becomes liberated at the time of the disintegration of the spirilla.

Before ending this discussion upon the subject of the local reaction produced by the extracted prophylactic, I wish to call attention to the fact that Hetsch and Kutscher,[25] who employed the method of inoculation of the free receptors in the typhoid bacilli prepared according to the method of Neisser and Shiga, reported that very marked inflammatory local reactions followed the injection of 0.5 cubic centimeter of the prophylactic in the inoculation of some of the German troops. Redness and swelling of the tissues occurred three hours after the injection. The inflammatory area about the point of inoculation became of a scarlet-red color, sharply circumscribed, resembling the inflammation frequently seen in erysipelas. These manifestations subsided after forty-eight hours, but the injections were not repeated because of the marked local reaction which occurred from the first inoculation.

I wish to emphasize that these results obtained with extracts of the typhoid bacilli are entirely contrary to those which we have encountered with the free receptors of the cholera organisms. In very numerous inoculations performed in Americans and natives of these Islands we have had abundant opportunity to observe the local reactions following the inoculation of the prophylactic I have described; none of these have been severe and the great majority have been very mild. Our injections have been made intramuscularly because of the quick absorption which occurs from the muscular tissues. Since the immunizing substances in the extracted prophylactic are either in solution or colloidal suspension,

[25] *Klin. Jahrbuch.* (1905), 14, 148, 156.

they apparently are in a condition in which they are capable of being much more easily absorbed than when they are injected bound to the bacterial cells. However this may be, with the cholera organism the local reactions are unquestionably less severe after inoculation with our prophylactic than from the injection of the living spirillum. I have received inoculations by both methods, and the suffering caused by the living organisms is much more marked.

SIZE OF THE DOSE AND STANDARDIZATION OF THE PROPHYLACTIC.

Most observers, in discussing the size of the dose in inoculations against cholera, agree that the immunity obtained is in proportion to the amount of the immunizing substances injected. This was shown very conclusively by my early experiments which have been referred to in this article. However, Friedberger and Moreschi combat this view and, as has been mentioned, believe that as high an immunity can be obtained from the intravenous injection of very small amounts of the cholera spirillum as from the subcutaneous injection of much larger ones. I have not had any experience with the intravenous inoculation of the cholera organism in such small quantities as Friedberger and Moreschi have employed. The experiments of Fischera and of Meinicke, Jaffé and Flemming do not confirm Friedberger and Moreschi's results, as has been mentioned in the discussion of the literature; moreover, they believe that *with such small quantities of the organism* the very favorable results obtained may have depended more upon the individual variation of the animal in regard to susceptibility and immunity than upon the amount injected. In relation to the size of the dose inoculated it may be recalled that Wright,[26] in his inoculations against typhoid fever in the English army, attempted to inject each individual with almost exactly the same number of killed typhoid bacilli. In order to accomplish this purpose he employed a twenty-four. hour broth culture of a known and proved strain of *Bacillus typhosus* and enumerated the number of bacteria in this culture by the ingenious blood counting method which he devised.

Leishman and Harrison,[27] in further pursuing the question of typhoid inoculation among the English troops, also attempted to standardize their vaccine by the procedure advanced by Wright. However, they found that in spite of all precautions, errors in the broth cultures of from 50 to 100 per cent in counts of the same films were by no means uncommon. Leishman and Harrison also spent some time in their efforts accurately to standardize their typhoid prophylactic (consisting of the killed typhoid organisms,) by estimating in addition with the assistance of Martin, the weight of the dried bacterial bodies in a

[26] *Brit. Med. Journ.* (1900), **1**, 122, and *Lancet* (1902), **2**, 11.
[27] *J. of Hyg.* (1905), **5**, 380.

measured quantity of vaccine and a correlation obtained from this weight and the number of bacteria as estimated by the living and dead counting methods. Even by all these methods it was impossible finally to arrive at an accurate standardization such as they apparently desired.

In my opinion these experiments performed in the standardization of either typhoid or cholera prophylactic are superfluous and unnecessary. The most practicable and accurate method I have been able to devise for the standardization of a prophylactic of this nature is the determination of the degree of immunization within certain limits which will usually be produced by approximately equal quantities of it in an animal. Evidently, exactly the same amount of immunity in a series of inoculated animals is practically never produced even though the dose injected is exactly the same, owing to the natural individual variations in the immunity of the animals.

Having determined upon an arbitrary unit of immunization and upon the minimum number of units of immunity a given volume of the vaccine must usually produce in animals, so that the inoculation of the same amount will give rise to the production of a satisfactory quantity of immune bodies in a few human beings, it is only necessary always to employ for man the amount of vaccine which will give rise in the animal to at least the minimum determined number of units of immunity. Since individual variations in immunity in human beings are so marked, it probably does not make any practical difference whether one individual is inoculated subcutaneously with a few hundred or perhaps even a thousand more killed organisms than is another one. Thus, while for adults the regular dose of the extracted prophylactic we employ is 2 cubic centimeters (which must give rise to at least 10,000 units of immunity in a rabbit), nevertheless, the results obtained in human beings inoculated with the same lot of prophylactic and with the same dose were surprisingly variable. Moreover, they conclusively demonstrated that nothing of practical importance was to be gained in attempting to inoculate each individual of a large number of people in a community with exactly the same quantity of receptors. I have observed two individuals, each inoculated with the same dose of the same fluid prophylactic, one of whom developed forty times as great a quantity of antibodies as the other. Such variations in the immunity produced in human beings after inoculation are not uncommon.[28] The same condi-

[28] Agglutinins do not invariably develop in the blood sera of human beings inoculated either with our prophylactic or with the living cholera organism. Two physicians were each inoculated with 2 cubic centimeters of the same lot of our prophylactic. After ten days the blood serum of one showed an agglutinative reaction in a dilution of 1: 700, while that of the other gave practically no agglutinative reaction. At about the same time I was inoculated with 3 oesen of the living cholera organism, and although a very marked local and general reaction was obtained, my blood serum ten days later showed practically no agglutinative reaction against the cholera spirillum.

tions are sometimes encountered in the immunization of monkeys, but they are less variable in the other lower animals.

Lamb and Forster [29] have proposed to adopt Wright's method of standardizing typhoid vaccine by determining the amount of the vaccine which will completely neutralize or remove the amboceptor content of a fixed quantity of normal goat's serum. However, since the binding power *in vitro* of the receptors in the vaccine, for the amboceptors of the serum may perhaps not exactly represent the immunizing power of the vaccine in the animal body, we have preferred to employ the method I have described. This method of standardization of the prophylactic by the units of immunity it gives rise to is obviously not an accurate one, but it is sufficiently accurate for all practical purposes. In standardizing our smallpox vaccine we regard the reaction obtained in a monkey following inoculation with it as the most important test of its efficacy; the exact degree of the reaction (which varies with the natural variation in the immunity of the animals) is not so important so long as a distinct reaction is obtained. In standardizing our cholera prophylactic we also seek to obtain a certain reaction in the serum of the rabbit, following its inoculation. The exact degree of the reaction obtained, provided a certain limit has been reached, is obviously of less importance, for the reason already given.

IMMUNIZING POWER AND VIRULENCE OF THE ORGANISM.

Another point about which some further discussion seems necessary is that of the immunizing power of the cholera organism to be chosen for the preparation of the prophylactic.

Pfeiffer, Friedberger [30] and I [31] found that, with cholera spirilla, a greater immunity was obtained with the more virulent organism. Pfeiffer and Friedberger employed four strains in their investigations. My experiments were carried on with two strains of cholera spirilla of widely different virulence and I was able conclusively to show that the virulent organism, upon inoculation, produced a higher immunity and at the same time bound a greater number of amboceptors in a cholera immune serum than did the avirulent one. At the time of the publication of these experiments I stated that "these conclusions apply to the two strains of cholera spirilla employed in the foregoing experiments. Whether they will also hold good with other strains of this spirillum or for micro-organisms in general must be decided by further experimental work." The experiments of Meinicke, Jaffé and Flemming seem conclusively to show that with different strains of the cholera spirillum

[29] *Scient. Mem. Med. and San. Off., India*, Calcutta (1906), **21**, 7.

[30] *Berl. Klin Wchnsch.* (1902), **39**, 581.

[31] *Publications of the Bureau of Government Laboratories, Biological Laboratory*, Manila (1904), **21**, 1, and *J. Exp. Med.* (1905), **7**, 229.

in vitro, the binding power of the organism for amboceptors in a cholera immune serum is independent of the virulence of the organism. In some instances they found that a virulent cholera culture was able to bind more amboceptors than an avirulent one, but in many instances the reverse was the case.

Their experiments referring to the relation between virulence and immunizing power are not numerous. This seems to me to be unfortunate, for by means of their method of examination and the large number of cultures which they studied, they were in a position to solve this problem conclusively. In fact, in the small number of experiments they record, only one instance is given in their table of results in which they found that 1/10 oese of a killed avirulent organism, when injected intravenously into a single rabbit, furnished a serum with a bactericidal value of 1:1,000, while 1/10 oese of a killed, highly virulent strain in another animal produced a bactericidal value of only 1:200. However, while the results of those experiments suggest that immunizing power is independent of virulence, nevertheless, in the inoculation with such small amounts of the organism, the individual variation in the immunity of an animal plays such an important rôle that it would not be prudent to draw any general conclusions from this single result. For example in this same series of experiments three other rabbits were inoculated with 1/10 oese of the cholera strain (number 74); one of these gave a serum of a bactericidal value of 1:2,000, one of 1:1,000, and one of 1:400. Therefore, it would seem that further experiments are necessary before we can reach a final conclusion on this subject. This question in particular is not settled in regard to the inoculation of the living organisms of different virulence, and of the relative immunity produced. I [32] recently performed some experiments with living plague bacilli of different virulence and found that the more virulent organism furnished the greater immunity. However, as I worked with but three strains of this bacillus my experiments also can not be considered as entirely conclusive for other strains of the plague organism. It seems possible that the coefficient of growth of the spirillum may play some part in the degree of cholera immunity produced; that is, the virulent organism may multiply more rapidly after inoculation than the less virulent one, as Gotsclich and Wiegand found in cultures; this need not necessarily imply that a greater volume of growth is obtained with the virulent organism, in fact the individual spirilla may be smaller in size than in the case of the avirulent culture. The larger forms of the spirillum, it has been observed, are much more common in cultures of the avirulent strains than they are in those of virulent ones. It must be recalled that Haffkine in connection with the question of virulence

[32] *This Journal, Sec. B., Med. Sci.* (1907), **2**, 187.

and immunizing power emphasizes the fact that the more virulent cholera organism produces the greater immunity, and MacFadyan [33] states that cholera cultures of high virulence yield the most toxic and cultures of low virulence the least toxic juices, while in those instances in which the virulence had been allowed to diminish to such an extent that 2 platinum loops of a culture did not kill a guinea pig, the toxicity of the juices suffered a corresponding drop, 0.5 and even 1 cubic centimeter failing to kill, whereas the animal succumbed to acute infection from 0.1 cubic centimeter from a virulent culture. This led MacFadyan to conclude that virulence and toxicity were intimately related as regards the cholera endotoxin, inasmuch as increased virulence implied increased toxicity and vice versa.

Therefore, in preparing our cholera prophylactic we select an organism which is known to possess high immunizing value and in addition one of maximum virulence.

THE SERUM TREATMENT OF CHOLERA.

Recently the question of the serum treatment of cholera has again attracted attention owing to the studies of Roux, Brau, and Denier, Kraus and MacFadyan, and before entering into a discussion of the subject I will briefly review their results.

Brau and Denier [34] found that they were able to obtain a very active toxin from the cholera vibrio by growing this organism in a special culture medium consisting of *bouillon Martin gélatiné,* 45 cubic centimeters, normal serum of the horse 45 cubic centimeters, defibrinated blood 10 cubic centimeters, heated to 60° C. for three hours. By growth of the organism upon this medium they were able to obtain the toxin regularly and in increased amount. After four days' development the cultures had become liquified; hæmolysis occurred after twenty-four hours; after seven days they were filtered through paper and then through a Chamberlain F. candle. Certain precautions are necessary in order to obtain the toxin in satisfactory amounts. They advise that the serum be heated at 60° C. for three hours in order to destroy the substances antagonistic to the development of the cholera vibrio. The thermostat must be kept at a constant temperature, variations even of 1° interfering with the production of the toxin; the optimum temperature was found to be between 38° and 39° C. It is also necessary for the cultures to be well aërated and shaken each day. Finally, the strain of cholera spirillum employed must not have been passed through animals, since such a passage diminished the toxic power of the organism with great rapidity.

Following this method they were able to obtain a cholera toxin with 26 cultures of vibrios isolated in Saigon, with two strains obtained from the Pasteur Institute, one of which was isolated in Bombay and the other in Nasik, and with three strains from Egypt. They concluded that a soluble toxin may be obtained from vibrios isolated from cholera stools and that the production of the toxin may be increased by cultivating the organisms in their special culture

[33] *Lancet* (1906), 171, 495.

[34] *Compt. rend. Acad. d. Sc.*, Par. (1905), 141, 397.

media. In the following year[35] the same authors called attention to the fact that this cholera toxin manifested its effect quickly and without a period of incubation when injected into an animal. Guinea pigs and rabbits could be immunized against the toxin so that they were able to resist two fatal doses injected at one time, and horses which had been inoculated intravenously at intervals of 6 months with ½ liter of the toxin, furnished a serum of which 0.02 cubic centimeter neutralized two fatal doses of the cholera toxin after a contact of thirty minutes *in vitro*. The serum also exerted antimicrobic, agglutinating and precipitating qualities. The cholera toxin was not destroyed by boiling and the boiled toxin produced as good a serum as the unboiled one. It was also found that the injection of cultures of the living cholera vibrio into the veins of a horse furnished an antitoxic serum which was even more active than that prepared with the soluble toxin. They admit that the cholera toxin appears to be analogous to the endotoxins of the pest and typhoid bacilli, although in their final conclusions they state that the organism produces a soluble toxin the action of which is rapid and without a period of incubation. They also believe that the cholera toxin contained in the exudates of the bacteria and that obtained in the liquid culture media, can not be distinguished. The authors in their last article emphasize some further precautions to be observed in order to secure a good production of the toxin. The media finally employed consisted of 20 cubic centimeters of normal serum of the horse plus 10 cubic centimeters of defibrinated blood. The serum and defibrinated blood must be at least three weeks old before use, as otherwise almost no production of toxin occurs.

In 1903–4, in studying the question of protective inoculation against cholera, I called attention to the fact that judging from my experiments "it would appear that the most advantageous method for the extraction of the intracellular toxins of the cholera spirillum would be the one which MacFadyan has recently applied to the typhoid bacillus with the same end in view. By this method the bacteria were ground up at the temperature of liquid air, the disintegration having occurred under conditions which precluded the possibility of chemical change."

MacFadyan[36] during the present year, 1906, has undertaken experiments of this nature with sterile juices obtained from the cholera organism. Toxic extracts were obtained from the most virulent cultures which killed guinea pigs acutely in doses of 0.1 to 0.05 cubic centimeter while 0.02 cubic centimeter rendered the animals ill. The endotoxin also exerted its action when injected subcutaneously in quantities of 1 and 2 cubic centimeters. Doses of 0.1 to 0.05 cubic centimeter killed rabbits on intravenous injection. The juices deteriorated in toxic power on keeping, and the latter was destroyed by heating at a temperature from 55° to 60° C. Goats were immunized with increasing doses of the endotoxin and a serum was obtained of which 0.002 cubic centimeter neutralized from three to four ascertained lethal doses of the endotoxin for a guinea pig. This property was not possessed by 1 cubic centimeter of normal serum.

Kraus,[37] in 1904, in working with a vibrio designated as "Nasik," was able to obtain a powerful toxin from filtered bouillon cultures of this organism. By heating to 50° C. its poisonous properties were destroyed. Kraus concluded that his organism was not a true cholera vibrio owing to its agglutinative, bactericidal, precipitating, and haemolytic properties. Since this time the same author[38]

[35] *Compt. rend. Acad. d. Sc.* Par. (1906), **142**, 728, and *Ann. d. l'inst. Pasteur* (1906), **20**, 578.

[36] *Lancet* (1906), **2**, 494.

[37] *Centrbl. f. Bakteriol. Orig.* (1904), **34**, 488.

[38] *Ibid*, (1906), **41**, 15, and *Wien. klin. Wchnsch.* (1906), **19**, 655.

has carried on extensive experiments with a number of different vibrios, which can not here be considered in detail. In his most recent article on the subject [39] he concludes that the cholera vibrio of Koch produces no haemotoxin either in buillon cultures or in goat's blood-agar plates. However, it gives rise to a toxin which is either produced by the spirillum only in the living organism, or also *in vitro*. The cholera poison is a true, soluble toxin and may be destroyed by antitoxin. It is to be differentiated from Pfeiffer's endogenous poison, which in the organism produces no antitoxin. Cholera is therefore an intoxication which is excited by a secreted, soluble toxin.

A study of Kraus' experiments does not seem to me entirely to justify his conclusions. Moreover, his results are not altogether in accord with those of Brau and Denier. Kraus distinguishes two kinds of soluble poisons in the different spirilla, one, which is the most potent, acutely acting toxin, such as that produced by the *Vibrio nasik,* and a large number of other not true cholera vibrios, and a second which is a slowly acting, poisonous substance encountered in filtrates of true cholera cultures and which he regards as the toxin which gives rise to the cholera symptoms observed in man. Brau and Denier state that the toxin they obtained from the cholera vibrio acts acutely and without an incubation period, and that they secured this toxin from the *Vibrio nasik,* as well as from many other undoubted cholera strains. The toxin with which they worked was not destroyed by boiling, while the one which Kraus obtained from the *Vibrio nasik* was destroyed at a temperature of 50° C. However, Brau and Denier in their last publication on this subject incline to the belief that they formerly encountered two toxins, one of which was destroyed by boiling and the other not. Kraus has apparently lost sight of the fact that MacFadyan has obtained an anti-endotoxic serum of such potency that 0.002 cubic centimeter protected a guinea pig against three lethal doses of the cholera endotoxin, while Brau and Denier found that 0.002 cubic centimeter of horse's cholera immune serum, the animal having received 0.5 liter of toxin intravenously, was able to neutralize but two fatal doses of the toxin after standing one-half hour *in vitro*. This serum did not follow the law of multiples, as 0.05 of a cubic centimeter was necessary to neutralize 3 lethal doses of toxin, while 1 cubic centimeter was required to neutralize 4 doses.

I demonstrated in 1903 that 0.2 cubic centimeter of a cholera anti-endotoxic serum would neutralize 4 lethal doses of toxin, when mixed immediately before inoculation. I also found, as MacFadyan has since done, that a temperature of 60° C. destroys most of this primary poison, or at least converts the toxin into toxoid. It would appear that the toxin which Kraus has obtained and which he designates as a secretion of the organism and as a soluble toxin, is none other than the one with which Brau and Denier, MacFadyan, and myself worked and that it should

[39] *Centrbl. f. Bakteriol.* Referate, (1906), **38,** Beil. 84.

rather be regarded as an endotoxin, for convincing evidence to the contrary at least has not yet been brought forward.

During the year, opportunity was afforded to study the antitoxic serum of Denier and to witness its practical application in the treatment of Asiatic cholera. A request having been made by Dr. Denier to carry on the experimental serum treatment of cholera in the Government cholera hospital in Manila, I was called upon to examine the sera and report upon them before this method of treatment was undertaken. The two sera which Dr. Denier employed were of a different nature. One serum designated as "A" was prepared by injecting the horse with the cholera toxin entirely free from the bacteria, and the second one, "B," was produced by injecting the horse with the living organisms. These sera were, upon examination, found to possess specific agglutinative and bactericidal properties, serum "B" showing a much higher value in this respect. No study was made of the neutralizing power of the sera for lethal amounts of the filtered cholera toxin. Guinea pigs inoculated with 1 cubic centimeter of serum "B" and at the same time with 1 or even 2 oesen of a cholera vibrio, of which the lethal dose was 1/10 oese, survived the inoculation; however, when they were inoculated with 5 oesen and 2 cubic centimeters of the serum, they invariably succumbed. Pfeiffer's phenomenon seemed to have been complete, as was shown by the postmortem examination of a number of these animals, since microscopic preparations from the exudate in the abdominal cavity showed no motile vibrios and the animals had apparently died rather from an intoxication than from an infection. However, these experiments obviously do not demonstrate whether death had occurred from the effect of the endotoxin contained in such a large amount of the spirillum (5 oesen) or from the effects of another soluble toxin.

Serum "B" was found to protect against larger doses of the living organism than serum "A" as was proved by testing the bactericidal power of the two sera. The bactericidal value of the sera was apparently, at all events so far as the living organisms were concerned, the most important factor in protecting the animals, at least up to a certain dose. In many of the animals which died and which had not received excessively large doses of the cholera spirillum, Pfeiffer's phenomenon was also found to be complete or almost so.

In all, 52 human cases of cholera were treated by Dr. Denier with the sera. In each instance a bacteriologic diagnosis of cholera was made by Dr. Denier and also by this laboratory, as was customary with all cases in the Government hospital. The injections of the sera were given intravenously and in large quantities, as much as 250 cubic centimeters in a liter of Hayem's solution being inoculated at a single dose. Following this primary inoculation, 100 cubic centimeters of serum were injected in an equal amount of saline solution every three hours until a reaction

on the part of the patient occurred. The average amount of serum given was from 300 to 500 cubic centimeters, but in one case 1,000 cubic centimeters were inoculated in twenty-four hours. The cases in the hospital were treated alternately with serum, that is, every other case admitted received this treatment. Dr. Denier remained at the hospital day and night and was indefatigable in his efforts to treat and care for the sick. The injections of the serum were usually given very shortly after the time of the admission of the case to the hospital. Obviously, the patients were frequently in collapse at the time of their arrival. Dr. Denier [40] has prepared the following table which at a glance shows the results of the serum treatment.

	Number of cases.	Cholera spirillum not isolated from the stools.	Dead.	Recovered.	Percentage of mortality.
Controls	21	3	13	5	72
Serum "A" antitoxic	16	1	11	4	75
Serum "B" antimicrobic	5		2	3	40

From this table it is evident, as Denier points out, that the cases which received the antitoxic serum were not benefited by it, the mortality being even higher than in the ones which received no serum. The number of cases which received the antimicrobic serum is too small to justify decided conclusions, although the mortality is much lower. Denier calls attention to the fact that liquid and frequent serous movements occurred shortly after the inoculations, with the patients who received intravenously a large amount of the serum in Hayem's fluid, these movements in volume approximately equaled that of the liquid injected. Therefore, he thought that possibly the antitoxic serum was not retained in the body for a sufficient length of time to accomplish its action and that it was perhaps excreted into the intestine and passed in the stool. He suggests that the injection of the serum might therefore, under proper aseptic conditions, be made with better results into the abdominal cavity. It has occurred to me that, if the inoculated serum was excreted into the intestine, more favorable results might perhaps be obtained, at least in the early cases, with a serum of higher bacteriolytic power, since, in the event of the excretion of the serum by the mucosa of the intestine, it would be brought into direct contact with the cholera spirillum. Probably such a serum would exert no favorable influences by its bacteriolytic properties in the later stages of the disease. It would

[40] Report à Monsieur le Gouveneur Général de l'Indo-Chine, Hanoi, Saigon, Oct. (1906.)

be interesting to ascertain whether better results could be noted in the treatment of cholera by the use of the antitoxic serum prepared according to the method recommended by MacFadyan, since he has, according to published reports, prepared the serum of the highest endotoxic power.[41]

As yet we have not by our own experiments, been convinced of the production in cultures of a *soluble* toxin by the cholera spirillum, even when it is freshly isolated from the cholera stool. On the other hand, it does not appear to me definitely to have been determined that the toxin which MacFadyan and others have obtained from the protoplasm of the organism, really is the toxin in the same condition in which it gives rise to all of the symptoms of the disease in man. At all events, it would appear that the cell juices which MacFadyan has isolated probably also, in addition to the pure specific cholera toxin, contain certain other poisonous substances. A more extensive routine study of the cholera vibrios isolated freshly from the cholera stools, as well as of the strains of *Bacillus dysenteriæ* encountered in dysentery fæces, in relation to the formation by them of soluble toxins, will be pursued in this laboratory as opportunity offers.

[41] In a personal communication from Dr. Denier I am informed that Dr. Besredka has recently succeeded in preparing an antitoxic cholera serum which possesses a much greater value than did the serum with which Dr. Denier performed his experiments in the treatment of human cases of this disease during the preceding year.

OBSERVATIONS UPON TREPONEMA PERTENUIS CASTELLANI OF YAWS AND THE EXPERIMENTAL PRODUCTION OF THE DISEASE IN MONKEYS.

By P. M. Ashburn and Charles F. Craig.[1]

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PART I.

HISTORICAL.

Schaudinn and Hoffman(3) in May, 1905, announced their discovery of a spiral-shaped parasite in the lesions of syphilis, which they named *Spirochæte pallida.*

As *spirochæte,* Cohn, 1872, is an amended spelling of *Spirochæta.* Ehrenberg, 1834, the name *Spirochæte pallida* became *Spirochæta pallida.* In the same year Vuillemin(4) selected *Spirochæta pallida* as the type of a new genus which he called *Spironema,* the organism found in syphilis thus becoming *Spironema pallidum,* a classification accepted by Schaudinn in 1905. Further investigation developed the fact that the name *Spironema* had been previously employed to designate a genus of mollusks, and accordingly could not be used in this connection. Stiles and Pfender(5) proposed the name *Microspironema pallidum* for the organism but before their publication appeared Schaudinn(6) had proposed the generic term *Treponema* and the specific name *Treponema pallidum,* Schaudinn, which is the correct name of the parasite of syphilis.

In February, 1905, Castellani(7) while investigating the etiology of yaws at Colombo, Ceylon, discovered spirochætæ in the serum of yaws lesions, one of which resembled very closely *Treponema pallidum* in its morphology. In the announcement of this discovery, which appeared in the *"Journal of the Ceylon Branch of the British Medical Association,"* June 17, 1905, he named the organism *Spirochæta pertenuis,* but as it undoubtedly belongs to the genus *Treponema,* the correct name is *Treponema pertenuis* Castellani. Several papers by this investigator have since appeared (8, 9, 10, 11, 12) dealing with the etiology of yaws and a few confirmatory reports of the presence in the lesions of yaws of *Treponema pertenuis.*

Wellman(13), in South Angola, Africa, was the first to confirm Castellani's observations, finding the organism in scrapings from yaws lesions in one case.

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He was not aware of Castellani's discovery at the time, July, 1905, so that his observations amount to an independent discovery of *Treponema pertenuis*, although the organism was first seen and described by Castellani. In a supplementary note regarding the *spirochætæ* found in yaws, Wellman says(14): "It is significant that this observation which has been spoken of as one of the most important discoveries of recent times, considering the fact that the *Spirochæta pallida* has been found by Schaudinn in syphilis, and considering the relation said to exist between yaws and syphilis (15) should have been made almost simultaneously in two such widely separated countries as Ceylon and West Africa."

Further confirmations of the presence of *Treponema pertenuis* Castellani in the lesions of yaws have been published by Powell(16), Borne(17) and MacLennan(18). Powell and McLennan found the organism in but one case, but Borne encountered the treponema in nine of eleven cases examined, and the latter wrote Castellani(19) that he had found them in forty-nine out of fifty-nine cases. Connor(20) was unable to demonstrate *Treponema pertenuis* in two cases of yaws occurring in Manipur State, India, using Leishman's stain, the method advocated by Castellani.

Description.—The following résumé is compiled from the published descriptions of the organism by Castellani. The treponema were found by him in the serum from the nonulcerated lesions and in smaller numbers in the ulcerated lesions of yaws.

The majority of the organisms are extremely delicate, though some individuals are thicker and stain more intensely, but all are thinner than "*refringens*" or other spirochætæ with the exception of *Treponema pallidum* Schaudinn. The length varies from a few μ to 18 or 20 or more. Both extremities are often pointed, but forms are met with presenting blunt extremities or one extremity blunt and the other pointed. Rarely, one extremity may show a pear-shaped expansion or a loop-like formation. The organisms are spiriliform, the number of waves in the spiral varying, but generally being numerous, uniform, and of small dimensions; sometimes an organism is observed having uniform, narrow waves for a portion of its length, the remainder being almost or quite straight. Two organisms may be attached together end to end, or twisted about one another. Castellani has seen forms indicating longitudinal division, two organisms lying parallel, close together and united at one end; he has also observed a few chromatoid points scattered irregularly in some organisms. With Leishman's or Giemsa's stain the organism takes a pale, reddish tint. Castellani also found, in very rare instances, a few oval or roundish bodies 5 to 6 μ in length, and 4 to 6 μ broad, staining purplish or bluish with Leishman's stain, and containing chromatin, collected at one point or scattered throughout the bodies. He suggests that these bodies may be a developmental stage of the *Treponema pertenuis*. In the open ulcerative sores of frambœsia Castellani found, along with *Treponema pertenuis*, three varieties of spirochætæ, as follows:

1. A thick, easily stained form, identical morphologically with *S. refringens* Schaudinn.

2. A thin, delicate form, with waves varying in size and number, and blunt at both ends. He named this organism *S. obtusa*.

3. A thin, delicate form, tapering at both ends, which he named *S. acuminata*.

Castellani found *Treponema pertenuis* present in the lesions of yaws in eleven of fourteen cases. Regarding its morphological resemblance and its relation to *Treponema pallidum* he said in 1906(21):

"The spirochætæ found in the non-open lesions and some of those found in open sores of yaws are, in my opinion, morphologically identical with the *S. pallida* of Schaudinn. This is also the opinion of Schaudinn himself who

very kindly has examined several of my specimens, but that if future investigation will prove that yaws is a spirochætæ disease, the yaws spirochæte will have to be considered to be biologically different from the spirochæte of syphilis."

Careful and frequent inquiries among the medical officers of the Army and civilian practitioners in Manila, during a period of almost a year, had but confirmed the impression gained from the literature (1 and 2), that yaws is a rare disease in the Philippine Islands, when we were shown some cases at Parañaque, through the courtesy of Dr. Luis Guerrero. At the time of our first visit there we saw four cases and at subsequent visits four others, while we were informed by the patients and their friends that the disease is very common throughout all the region about Parañaque, and we have since heard of it as common in certain towns of Tarlac Province, Luzon, and in the neighborhood of Parang-Parang, Mindanao, and it is quite probable that it is frequently seen and well known by the natives in most parts of the Archipelago.

We have also seen five cases in San Lazaro Hospital, Manila, all in lepers. We have not had an opportunity to treat any case that we have seen, but we have examined all and in ten we looked for *Treponema pertenuis*, finding it in all of them.

Our examinations of yaws cases, which have been made at relatively infrequent intervals for the reason that we had none under our immediate control and supervision, embraced inquiries into the clinical manifestations of the disease. In this regard they brought out nothing new that is important and the description which we might give of the clinical appearance of the disease would not differ greatly from those of most recent authors and even from those of a century ago by Winterbottom and Bateman(22), except that we think the large, ulcerative lesions are probably due to secondary infections and should not be credited to pure yaws, any more than suppuration in syphilitic lesions should be attributed to *Treponema pallidum*.

The observations which we shall discuss herein consist principally, then, of studies of the fresh and stained serous exudate from yaws lesions, which contained the *Treponema pertenuis*, as described by Castellani and others. As the serum presented nothing peculiar or characteristic of yaws except the treponema, the great bulk of our work consisted in observations on that parasite. These observations were made on three varieties of preparations of the serum.

METHODS OF EXAMINATION.

A. Stained smears.—These were prepared by removing the yellowish, beeswax-like tops from the papillomatous lesions, either by pulling them off entire or by washing them off by friction with wet gauze, and taking on the end of a slide a bit of the clear serum which then exudes from the lesion and making a very thin smear of it across a thoroughly cleaned slide. Preparations so made were then stained with either Wright's or Giemsa's stain, preferably the latter.

A more profuse flow of serum is obtained if the crust or cap of the swelling

be washed away, as the friction necessary in this process probably causes an increased flow of blood to the lesion; at any rate, a remarkably profuse flow is so obtained. On the other hand, if the cap be merely pulled off, the serous flow may be very slight and it only becomes profuse when the papilloma is rubbed with the end of a slide or with a piece of gauze, and even then it is not so abundant and so free from cells as the serum obtained by the first method.

It is remarkable how clean and how free from body cells and bacteria the slides thus made may be.

B. Fresh serum.—This was obtained in the same way as that for staining, except that it was generally allowed to flow into capillary tubes, whence it was blown out upon slides and diluted or not, as seemed desirable, with a small amount of normal saline solution. It was then covered with a thin cover glass, the glass ringed with vaseline and examination made with a high power.

C.—Capillary tube preparations were made as indicated above, the tubes being sealed when filled and kept at room temperature (usually about 30° centigrade) for variable lengths of time, when they were broken, the contents blown out and examined stained or unstained, or both.

In addition to the examinations made of the serum, in the ways just indicated, we excised two papillomata and sectioned and stained them by Levaditi's method. We were unable to demonstrate the *Treponema* in these preparations, but in sections similarly stained and sent to us by Captain Russell, acting curator of the Army Medical Museum, they are seen in great numbers, lying among the epithelial cells, but less numerous among the deeper layers of these. The organisms are in many instances aggregated into clumps similar to those obtained in the capillary tubes. The cells among which the organisms are found always show signs of degeneration, loss of outline, indistinctness of nuclei, and vacuolation. Such areas are localized and present the appearance of lacunæ of degeneration.

DESCRIPTION OF TREPONEMA PERTENUIS CASTELLANI.

a. Morphology.—The morphology of the treponema may be very briefly described by the statement that it is indistinguishable, so far as we can determine, from that of *Treponema pallidum*. In shape, size, staining reactions, appearance of ends, etc., the two are similar, and neither we nor the many medical men and investigators to whom we have shown the organisms and whose opinion we have sought, are able to differentiate them.

In length *Treponema pertenuis* varies considerably, some short forms not being longer than about 4 μ. It is possible that other forms may be even shorter than this, but if so they are not recognizable as treponema. More commonly they are about 10 to 12 μ in length, while individuals are even longer. Occasionally very long forms are seen, 20 and 25 or very rarely even 30 μ, but whether these are single individuals, or multiplying or agglutinating forms in which two individuals are joined end to end, we can not yet determine.

The width of the organism is so very slight that we are unable to measure it with exactness. We estimate its width as probably varying from one-sixth to one-half of a micron. If the line of a filar micrometer is brought as near to one side of a loop of the treponema as is possible

without covering the latter, and the line of the instrument be moved $\frac{1}{6}$ or $\frac{1}{4}$ μ toward the treponema, the side of the loop will be hidden in most instances. Whether it will be completely covered, or more than covered by the line, we can not say. We can not be more definite than to say that in our opinion the width of the organism is not far from 0.25 μ, but it may be either greater or less. The length of the spiral turns averages very close to 1.5 μ, measured from crest to crest. When we first began to study the organism we thought that *Treponema pertenuis* was probably a trifle wider and a trifle more open in its curves than *Treponema pallidum*, and we yet think that this may possibly be true for the average of large numbers, but our average measurements are the same for both, and there is no form that we have seen which we felt justified in designating as either *pallidum* or *pertenuis*, one and not the other, unless we knew the source from which it was derived.

The curves of both species of treponema vary somewhat in width and regularity, but these variations are not peculiar to or even much more common for either species. In general, the curves are fine, about 1 to 1.2 μ in depth, regular and rather rigid. The last-named character is especially noticeable in unstained, fluid preparations. Here the organisms are seen as fine and fairly rigid spirals, usually straight or almost so. The appearance is the miniature of that produced by a long spiral wire spring. Such a spring may be bent by slight pressures, but it at once resumes its straight form when the pressure is relieved, and in either the straight or the bent form it retains its spiral turns.

This description applies particularly to fluid preparations from a few hours to a few days old. In quite fresh preparations the treponema can not usually be seen, or if seen, recognized. Flashes of very motile organisms may be observed, and it is a fair presumption that some or all of them may be treponema, but the motion is so rapid and the glimpse of the organism so fleeting that no deductions can be drawn as to morphological characteristics.

It is important to note that in fluid preparations the morphology of the organisms is much more regular than in stained ones, and it is therefore probable that many of the variations in the latter class may be due to the drying and staining process. This statement applies to our experience with both *pallidum* and *pertenuis* and we think that it lessens the value of the deductions based solely on the morphology of stained specimens.

However, since the described morphology of both *Treponema pallidum* and *Treponema pertenuis* heretofore rested almost entirely on the descriptions of stained specimens, it is well to consider such specimens here; but it should be borne in mind, that no matter how many shapes, sizes and forms the stained organisms may show, there is not one of them which can not be imitated by the use of the spiral wire spring to which we likened

those in the wet preparations, if the spring be subjected to forces analogous to those acting on the treponema during the making and drying of the smear.

The stained forms, presenting many variations as to size and shape, may be most conveniently described by dividing them into types or classes. While the length and number of curves vary greatly, the examination of a large number of *Treponema pertenuis* shows the average number of curves to be about eight. Seventeen is the greatest number counted on one organism, two the smallest. Many individuals show only four or five turns, only a few have more than twelve. All types, shapes and sizes stain with difficulty, showing best with Giemsa's stain, which gives them a pinkish-violet color.

Type A (see Pl. IV) is probably the most common stained type of *Treponema pertenuis,* as it is of *Treponema pallidum.* This may be said to be the classical type of the latter, but the other forms to be described for *pertenuis* may also be found for it.

This type is usually straight, or but slightly bent; it shows regular and even curves which are very fine, and terminates in narrow pointed ends which have been interpreted as flagella. It stains evenly throughout, although the finely pointed ends show less distinctly than the main part, probably because of their size.

Type B is likewise very common, and differs from A in having a straight and usually thin portion in some part of its course, frequently near the middle. This appearance has been variously interpreted by different observers; principally as a union of two organisms by their flagella in beginning agglutination, as an incomplete separation of individuals resulting from longitudinal division, or as beginning or incomplete transverse division.

A spiral wire spring may be made to present a similar appearance if one or more of its coils is straightened out by traction and pressure.

Type C corresponds most closely with that we consider the unaltered form as seen in wet preparations. It corresponds to type A except that instead of terminating in finely pointed ends, it shows a dot or little knob at one end or often one at each end. This is the appearance seen almost uniformly in wet preparations and we think that it is due to a tight curling of the ends, such as is seen on the shoots of many young plants. This curl may straighten out under the influence of age or drying, and the end may then appear as a finely attenuated point or flagellum.

Type D is also common and differs from A and C in that one or both ends, instead of showing a finely attenuated point or blunt dot, show a ring.

This ring frequently appears thicker and heavier than the main part of the body. With the spiral wire we may obtain a similar picture if we

turn up an end or the ends of the spiral so as to look down through part of it and view another part from the side. That such bends should frequently be caused by the spreading and drying of the serum is readily to be believed for the reason that we often see the same thing entirely in profile, that is, the spiral turns are still preserved, but the whole spiral so bent as to form two or three sides of a quadrangle.

Type E may present the terminal features of any of the above but it also shows in its continuity a complete, circular loop, or more than one. The spiral wire assumes a similar appearance if one turn be pressed down or back.

Type F represents a short, loosely curved organism attached to a ring or two rings, one at either end. This, we think, differs from D only in the proportions of the spiral viewed from the end, and in the fact that the part viewed in profile has the regularity of its curves more altered by pressure or drying.

Type G represents various irregular forms which differ from the more characteristic individuals in form only, not in size or staining power. It is possible to produce all of them by pressure or traction applied in various ways to the wire spiral.

Type H embraces the individuals which show dots in some part of their continuity. These have been referred to by various writers as chromatin dots, as representing nucleus, blepharoplast, etc. Whether or not they be such, or are merely kinks or twists in the organism, we do not know. We are unable to determine any constancy in the frequency, number, or localization of their occurrence, and an analogous appearance may be caused in the wire coil by kinking or twisting it.

These various types may be found pure or in a great variety of combinations, such as B C, G D, C D H, H G, A H, etc.

Types A, B and C are seen in the wet preparations, and of the three, C is by far the most common. It is unusual to see in such preparations any individuals which do not show the knob-like ends and the regular curves throughout their length. The other forms D, E, F, G, and H we have rarely or never seen in wet preparations, but occasionally we have observed individuals, particularly in old ones, which presented a somewhat beaded appearance that might represent type H of the stained specimens.

MOTILITY.

In neither fresh nor stained specimens have we seen anything that we interpreted as an undulating membrane, nor anything that was differentiated as a flagellum in the distinct manner in which flagella are differentiated on certain bacteria or *trypanosomata*. The motility of the organisms as seen in wet preparations probably varies from the extremely active movement already mentioned, which permits one to see a flash of

glancing light but no more, to a very sluggish motion which so closely simulates entire passivity as to leave the observer in serious doubt as to whether any is present, other than that due to currents in the serum.

When motion has ceased to a sufficient degree to permit of the organism being well seen and clearly identified, it is always slow. It consists of a slight rotation on the axis of the spiral representing the appearance of a corkscrew movement, and a mild and gentle waving and bending of the entire organism. These two varieties of motion combined, cause the treponema to pass across the field, to rise and sink, necessitating much change of focus, or, if one end of the organism it attached to the cover glass, or to foreign matter in the field, as is frequently the case, to lash or to swing in an indolent manner from this fixed point.

This motion and what we consider the common form of the organism may best be observed in capillary tube preparations about one day old. In that length of time the accompanying bacteria have usually not multiplied so greatly as to occupy the large part of the field they do later, while the treponema have ceased to move actively, and have usually increased in number and are readily found. In these preparations, or stained ones made from them, what are most commonly called the dividing forms are more frequently seen.

DIVIDING FORMS.

For ease of description we may designate these forms as additional types I, K, L and M.

Type I is fairly common and, as indicated by Plate IV shows some variety in the arrangement of its component parts. Essentially it consists of two or more spirals which are attached one to another by their ends. In some, this attachment is such as to be almost or quite indistinguishable from type B.

Type K is also quite common and differs from I in that the attachment is firmer and involves a greater part of the length of the parasite. It is as though the wire of the spiral had been split throughout a quarter, a half, or three quarters of its length, the turns being preserved. However, the two sections of the spiral are frequently unequal in length. The appearance presented in such cases has been figured by Siedlecki and Krystalowicz(23) as representing conjugation.

Type L is probably the most striking and pretty form to be seen. Here the two component parts are intertwined throughout their length, the two ends at one extremity, however, being free. Occasionally all four ends are free, but more commonly those at one extremity are fused, or they take their origin from a common dot or knob.

These types, I, K, and L, have usually been considered indicative of longitudinal division, and they so appear to us.

However, some writers, particularly Novy and Knapp(24), have considered it possible that division of *Treponema* may be by transverse fission, and type M, which is very rare, would seem to be an example. In this type we see what looks like one organism but it shows a break in its continuity. More than one break rarely occurs. Whether these apparent breaks are really such, or are merely artefacts, we do not know. They usually appear like the latter. We have seen the appearance indicated both in stained preparations and in photomicrographs, but not in wet specimens.

VIABILITY.

We have found treponema which showed slight motion and preserved their forms in a capillary tube preparation of serum made thirty-four days prior to the time of its examination; apparently the organisms were alive but we made no inoculations with them. The bacteria, which had been very numerous in other preparations made at the time, but not kept so long, had so far as we could see all died, leaving a pure culture of *Treponema pertenuis.*

Undoubted motion is preserved by organisms in tube preparations for a period of several days, although it is always sluggish after a few hours and often after one hour or less.

CULTIVATION.

At the suggestion of Dr. Miyajima we studied capillary tube preparations of the serum from yaws lesions, in the hope of obtaining agglutination of the treponema, as Dr. Miyajima said that he had obtained it in similar preparations from chancres. Our hopes in that direction were speedly realized, as we obtained marked clumping in the first one we made. These tubes were examined on the second, third and fourth days.

We have not since had an opportunity to discuss the matter with Dr. Miyajima, and therefore are not quite sure as to what he meant by the term "agglutination." If he used it as meaning merely the aggregation of already existing organisms into clumps, we think that his statement was not sufficiently broad, as it is our opinion that the numbers of treponema in such preparations are greatly increased after one day to a week.

Usually, the increase is manifest within 24 hours; however, at times, it takes place more slowly and may only become well marked after several days. The increase in numbers and the clumping are both almost, but not quite, constant; of the two increase is, in our opinion, the more so. We obtained a similar result in one of the two cases of syphilis in which we made the same experiment with serum from the chancre.

It is difficult to determine accurately whether a smear preparation

containing scattered treponema has as many organisms as another preparation in which they are in clumps. Novy and Knapp, who describe agglutination of *Spirillum obermeieri* say that in the case of that organism there is no increase in number. Counts being out of the question, the observers' estimate must be relied upon. Our belief, based on many examinations of sera made on the first, second, and third days, etc., is that in most instances *Treponema pertenuis* multiplies greatly when kept in the serum in capillary tubes, and in some instances the increase seems to occur without the agglutination. Our opinion that the organisms multiply in such preparations is based not only on the greater number of them found in the tube preparations as compared with smears made at the same time, but also on the appearance of the clumps and the great preponderance of what we consider dividing forms as described under types I, K and L. The nature of some such clumps and dividing forms are shown in the accompanying photomicrographs. (Pl. 1, figs. 5 and 6.)

Exceptionally, neither multiplication nor agglutination developed in the tubes, and the failures, while infrequent, are somewhat irregular and do not admit of what we consider a thoroughly satisfactory explanation. However, in general terms, we are of the opinion that they are due to one or both of the following reasons:

First. Variability in the immunity of the yaws patient and in the amount of antibodies contained in the serum; possibly the variability of immunity may even be local, or toxins elaborated by bacteria may be present locally to inhibit the growth. An interesting observation which may bear on this point was made in a case of syphilis.

Serum from a chancre was taken up in tubes on Monday. The chancre was then washed with bichloride solution and dressed with calomel. On Tuesday it was thoroughly cleaned and more serum placed in tubes. The tubes of Monday showed marked increase and clumping of treponema, those of Tuesday, neither. The patient was not taking any general treatment.

Second. Variability in the bacterial content of the tubes and consequently in their content of soluble toxins.

While it is readily possible to make fresh smear preparations of yaws serum which show very few or no bacteria, it has not been possible, in our experience, to obtain a serum really free from these organisms, and in the sealed tubes they multiply enormously. However, the fact that both multiplication and clumping occur in the great majority of tubes encouraged us in the idea that we might cultivate the treponema indefinitely, and as the favorable medium seemed to be the serum of susceptible persons or animals, we endeavored to obtain growth on monkey blood and on ascitic fluid from a patient who was suffering from cirrhosis of the liver who also gave a history of syphilis. The ascitic fluid was

used in two ways, plain and heated to 60° C. for thirty minutes to destroy complement. In none of these media did we obtain any growth of the treponema, although bacteria developed in all.

However, we did not have the opportunity to repeat these experiments, and we are not convinced that cultivation of the treponema is impossible.

PATHOGENESIS.

It has been stated by different authors that yaws is inoculable on lower animals, notably cats and monkeys, and that it is inoculable from person to person. We have made no inoculation experiments on persons, and none on lower animals other than monkeys (*Cynomolgus philippinensis* Geoff.). Of these we inoculated five, using serum from the yaws of three different patients. All five of the monkeys developed yaws lesions of a sufficiently characteristic appearance to permit of diagnosis based on that feature alone.

In addition to this typical appearance we found *Treponema pertenuis* in all of the lesions. The organisms did not differ in any demonstrable way from those seen in serum from human lesions. In numbers, measurements, staining reactions, shape and motion they were similar.

However, the monkeys did not show the secondary lesions of a generalized infection, nor could we, in the instance in which we tried it, induce yaws in other monkeys by inoculating them with the blood or splenic juice of an infected animal. The yaws lesion did spread, and in that way give rise to what might be termed secondary lesions, but this was always by continuity and we observed no evidence to make us think that it was ever through a general blood or lymph infection.

BIOLOGICAL POSITION OF TREPONEMA PERTENUIS.

We see no reason to doubt that the biological position of *Treponema pertenuis* is as close to that of *Treponema pallidum* as one species may be to another. The almost overwhelming weight of scientific opinion at the present time seems to leave the latter organism where Schaudinn placed it, among the Protozoa.

However, its protozoal nature is not universally accepted, and probably will not be for some time to come. Our opinion is that both organisms are protozoal, but while so eminent a zoölogist as Stiles(25) concedes to others the right to regard *pallidum* as of vegetable nature, we feel that we may safely grant the same latitude in respect to *Treponema pertenuis*. What we believe to be more immediately important and more easily determinable are the following propositions:

First. That *Treponema pertenuis* is constantly found in the serum from yaws lesions.

Second. That it can, at the present time, be differentiated from *Treponemà pallidum* only by the consideration of the lesion from which it is obtained, or by the inoculation of certain animals.

Third. That its many forms in stained preparations are all explainable on the supposition that it is a regular spiral, often deformed by the forces or processes concerned in the spreading, drying and staining of the smears.

Fourth. That, as will be shown more fully in Part II of this paper, the inoculation of serum containing this organism causes yaws in monkeys, and that the organism is again found in the lesions of the inoculated animals.

Fifth. That *Treponema pertenuis* is the cause of yaws.

PART II.

THE EXPERIMENTAL PRODUCTION OF YAWS IN MONKEYS.

Historical.—The literature relating to the production of frambœsia in monkeys by the inoculation of material from the lesions of the disease is very limited and so far as we have been able to determine Neisser, Baerman and Halberstädter, working together in Batavia, Java, and Castellani, in Colombo, Ceylon, have been the only investigators to produce the disease in these animals. To Castellani belongs the credit of demonstrating *Treponema pertenuis* in the experimental lesions in monkeys, the other investigators mentioned not searching for the organism, although, in their report they mention Castellani's discovery of a spirochæta in the lesions in man.

Neisser, Baerman and Halberstädter (26) inoculated seven monkeys with serum from yaws papules, the inoculation being made upon the breast and over the eyebrow, by rubbing the infective material into small abrasions in the regions mentioned. Frambœsia developed in all of the animals, the incubation period varying from thirteen to fourteen days in two *Gibbons*, to 96 days in *Macaccus*. In the latter, five in all, the incubation period was twenty-two, thirty-one, sixty-five, ninety-one and ninety-six days, respectively. So-called secondary lesions developed in three of the animals, forty, forty-nine and seventy days after the primary lesions had appeared, the authors stating that the secondary lesions always appeared upon the site of the initial one and extended in a serpiginous manner into the surrounding skin. They did not observe a generalized eruption in any of the animals. They also inoculated seven monkeys (*Macacus nemestrin, M. niger* and *M. cynomolgus*) with material from yaws papules in monkeys suffering from the disease. In only one of these animals (*M. niger*) inoculated from a *M. nemestrin,* did the disease develop after an incubation period of thirty-four days.

The authors then endeavored to produce the disease in monkeys by subcutaneous inoculation of a mixture of splenic juice, bone marrow and lymph glands from a *Gibbon* suffering from yaws. They injected three *M. cynomolgus,* with negative results in all. Inoculation of three monkeys of the same species with splenic pulp and three with the bone marrow from an infected *M. cynomolgus,* resulted in one of the three inoculated with bone marrow developing frambœsia after an incubation period of forty-four days.

These investigators also demonstrated that monkeys successfully inoculated with syphilis developed frambœsia upon inoculation. In one instance a monkey (*M. niger*) was inoculated upon April 17 with syphilis and developed the primary syphilitic lesion upon May 13. On May 28 the same monkey was inoculated with

frambœsia and the typical yaws papule developed at the site of inoculation upon August 1. Another monkey (*M. cynomolgus*) was inoculated September 23 with frambœsia, and on October 25 with syphilis. Upon November 8 a typical yaws papule appeared at the site of inoculation, while on November 15 the characteristic syphilitic lesion appeared.

From their experiments the authors mentioned draw the following conclusions:

1. Frambœsia is inoculable from man to higher and lower apes.

2. Frambœsia is inoculable from ape to ape.

3. Infection in monkeys following inoculation of the bone marrow proves that frambœsia is a general and not a local disease.

4. Apes infected with syphilis are susceptible to frambœsia.

Castellani (27) inoculated four Ceylon monkeys (species not given) with frambœsia. Only one of these developed the disease after an incubation period of nineteen days. A small papule, which enlarged slowly and became covered with a crust, developed at the site of inoculation. Two months later, the original papule being still present, four others appeared, two upon the forehead near the first lesion, and two upon the upper lip; one of these papules disappeared in a few days, but the other three enlarged slightly, became moist and a yellowish crust formed over each. At the end of three months all of the lesions had healed. In the scrapings from the lesions, *Treponema pertenuis* was demonstrated repeatedly.

Six weeks after the disappearance of the yaws lesions this monkey was inoculated with syphilis, and sixteen days later a typical syphilitic lesion developed, accompanied by general glandular enlargement.

The positive results obtained by the investigators whose work we have briefly reviewed, led us to repeat partially their experiments with a view of determining if the native monkey of the Philippines, *Cynomolgus philippinensis* Geoff., could be infected with frambœsia, and of adding, if possible, something to our knowledge concerning the disease as observed in these animals and the relation of *Treponema pertenuis* to the lesions produced by experimental inoculation. While our observations can not be considered as completed, we believe the results so far obtained are of interest and should be put upon record. In the main, our work has confirmed the results of the above mentioned investigators and we believe we are justified in asserting that frambœsia is easily inoculable from man to monkeys and that *Treponema pertenuis* is constantly present in the active experimental lesions and stands in a causal relationship to them.

Material and methods.—The monkeys used in our experiments were all *Cynomolgus philippinensis* Geoff., the common native monkey of the Philippine Islands. We have experimented with eleven monkeys, the inoculations in such animals being both by the subcutaneous pocket method and by vaccination, that is, rubbing a little of the yaws serum into slight abrasions upon the skin. The site of inoculation was generally the skin of the abdomen and forehead, but the inside of the thigh was used in inoculating with syphilitic serum. The method by vaccination proved, in our experience, slightly more successful than the subcutaneous pocket method, but it is probable that if a larger number of animals were used there would be found to be no difference in the results obtained. In every instance of successful inoculation, the slight wounds healed rapidly and the site of inoculation appeared normal until the development of the yaws papules at periods varying from sixteen to thirty-five or forty-five days after the inoculation. The serum used in making the inoculations was obtained in the manner already described for securing smears for staining.

In searching for *Treponema pertenuis* in the lesions in monkeys the same methods of securing specimens of the serum and staining them were used as have been already described in Part I of this report.

We also endeavored to secure cultures of *Treponema pertenuis* from the lesions in monkeys, using methods similar to those employed in our endeavors to cultivate the organism from human lesions.

In considering the experimental production of frambœsia in these animals we were desirous of investigating many problems intimately connected with the subject, aside from the mere successful result of inoculation, and while we have attempted to solve some of them, we do not feel justified as yet in expressing an opinion regarding our results in certain directions. This is especially true of our experiments regarding re-infection and the local or general nature of the disease as it is observed in monkeys in general, for our experiments in these directions are too few to be of definite value, although they are suggestive. The following protocols of our inoculations include those already completed and those in which it is too early as yet to predict the result.

PROTOCOLS OF THE EXPERIMENTS.

Monkey No. 1 (3070).—This monkey was inoculated on February 16, 1907, with serum from a typical yaws papule on a young Filipina girl. A subcutaneous pocket inoculation was made in the skin of the abdomen, and some of the serum was rubbed into a scarified area over the left eyebrow. Smears of the serum, prepared at the time the inoculation was made, showed numerous examples of *Treponema pertenuis*. The inoculation wound healed rapidly and the animal appeared normal until March 4, when a small papule covered with a yellowish crust was noticed at the point of the inoculation upon the abdomen; the crust was removed and smears made from the serum which exuded from the minute granulations. An examination of these smears demonstrated the presence of *Treponema pertenuis* in large numbers. The period of incubation was about sixteen days. Upon March 8, a small, crusted papule had appeared at the point of inoculation over the left eyebrow and smears of serum from this lesion also showed *Treponema pertenuis*. Both the lesions enlarged slowly, especially the abdominal one, and healing in the center, extended in a circular manner into the surrounding healthy skin. The lesion upon the head had disappeared by May 14, but the abdominal lesion persisted until May 28. Duration of the disease, eighty-two days. On May 15, this animal, still showing a yaws papilloma upon the abdomen, was inoculated with serum from a chancre which contained numerous *Treponema pallidum*. A subcutaneous pocket inoculation was made in the skin of the abdomen and in addition some of the serum from the chancre was rubbed into an abrasion upon the inside of the left thigh. No results have followed these inoculations to date, June 30, 1907.

Monkey No. 2 (3071).—On February 16, 1907, this animal was inoculated subcutaneously on the abdomen and through an abrasion over the left eyebrow with serum from a yaws tubercle from the same case as monkey No. 1 (3070). On March 8, a small, crusted papule was observed at the site of inoculation on the head, which gradually enlarged until it reached the size of a small hazelnut; a typical, yellowish crust developed, which upon removal disclosed the characteristic, pink, granulating surface of a yaws papil-

loma. Examination of the serum from the lesion demonstrated repeatedly the presence of *Treponema pertenuis*. The incubation period in this case was twenty days. The lesion had entirely disappeared on May 2, thus making the duration of the disease fifty-six days. On May 16 this animal was reinoculated with yaws serum through a subcutaneous pocket upon the abdomen and an abrasion over the right eyebrow. No lesion has appeared to date, June 30, upon the abdomen, but the inoculation wound over the right eyebrow suppurated and a deeply excavated ulcer resulted. Repeated examinations of the material from the ulcer have always resulted negatively for *Treponema pertenuis*.

Monkey No. 3 (3072).—This animal was inoculated February 26, 1907, with serum from a yaws tubercle on a native woman, the inoculation being made upon the abdomen and over the left eyebrow in the manner described. The serum used contained many examples of *Treponema pertenuis*. On March 8 after an incubation period of twenty days, a small, crusted papule was noticed at the site of inoculation upon both the abdomen and the head. Both lesions enlarged, became covered with a typical crust, and the examinations, which were made repeatedly, were always positive for *Treponema pertenuis*. This monkey did not stand close confinement well, became weaker and weaker, and was chloroformed on March 18, ten days after the appearance of the yaws lesion. At autopsy the viscera appeared normal, but the cervical and inguinal glands were slightly enlarged. The yaws tubercle upon the abdomen measured 1 by 0.75 centimeters, was considerably raised above the surrounding skin and covered with a yellowish crust. The lesion upon the head was 1.5 by 1 centimeter, and was very typical of the yaws papilloma as seen in man. The pathologic material was handed to Dr. H. T. Marshall, pathologist of the Bureau of Science, for examination.

Monkey No. 4 (3073) was inoculated February 26, 1907, upon the abdomen and over the left eyebrow in the manner described, with serum from a yaws tubercle in a native woman. Upon March 8, twenty days after inoculation, a typical yaws papule developed at the site of vaccination upon the head. This lesion enlarged slightly, became covered with the characteristic crust, and the examination of the serum from the granulation tissue revealed upon the removal of the crust showed the presence, repeatedly, of a few *Treponema pertenuis*. By April 16, the lesion had healed, the duration of the disease being about thirty-nine days. On May 15, this animal was inoculated upon the abdomen and right thigh with serum from a chancre showing many examples of *Treponema pallidum*. No results have been obtained from this experiment to date, June 30, 1907.

Monkey No. 5 (A).—This animal was inoculated April 10, 1907, upon the abdomen and right eyebrow, with serum from a yaws tubercle in a leper woman, the inoculation upon the head being subcutaneous, upon the abdomen by rubbing the serum into a slight abrasion. No lesion appeared upon this monkey until May 29, when a well-developed papule about 1 centimeter in diameter and covered with a thick, yellowish crust was observed. This lesion had evidently existed for several days, so that the incubation period is uncertain, probably between thirty-five and forty-five days. Upon removal of the crust the typical pink, raspberry-like growth was well marked and examination of the serum from the lesion demonstrated *Treponema pertenuis*. In this case the lesion enlarged but slightly and by June 12 had disappeared, the duration of the disease being about two weeks.

Monkey No. 6 (3109).—This monkey was inoculated on March 18 by the subcutaneous pocket method upon the abdomen and through an abrasion upon the forehead, with blood from the heart of monkey No. 3 (3072), obtained at the time of autopsy. No lesions have developed in this animal to date, June 30, 1907.

Monkey No. 7 (3110).—Was inoculated March 18, 1907, in the same manner

as monkey No. 6 (3109) with splenic juice from monkey No. 3 (3072) obtained at autopsy. No lesions have developed in this animal to date, June 30, 1907, but there is marked enlargement of the inguinal lymphatic glands.

Monkey No. 8 (3111).—Inoculated as above with serum from the yaws lesion upon head of monkey No. 3 (3072) obtained at the time of autopsy, March 18. This animal was in a weakened condition from continued confinement at the time of inoculation and died on April 6, nineteen days after the inoculation. No lesions of yaws had appeared at the time of death and the autopsy did not show anything of interest beyond enlargement of the spleen, liver, kidneys and the lymphatics of the abdomen.

Monkey No. 9 (B).—This animal was inoculated April 13 in the manner already described with yaws serum from a leper woman, the serum having been kept in a glass capillary tube for three days. No lesions have appeared in this monkey to date, June 30, 1907.

Monkey No. 10 (C).—Inoculated through a subcutaneous pocket upon the abdomen May 15 and through an abrasion upon the inside of left thigh, with serum from a chancre, showing very numerous examples of *Treponema pertenuis*. No lesions have appeared to date, June 30, 1907.

Monkey No. 11 (D).—Inoculated May 15 in the same manner as monkey No. 10 (C) with serum from a chancre showing the presence of *Treponema pallidum*. No lesions have appeared in this monkey to date, June 30, 1907.

The two latter animals were used as controls to our inoculation of syphilis in yaws monkeys No. 1 (3070) and No. 4 (3073).

SUMMARY.

The protocols given show that, in all, eleven monkeys have been used in our experimental work. Of these, five were inoculated directly with serum from human yaws lesions; one with serum from a human lesion, the serum having been kept in a glass capillary tube for three days; one with blood from the heart of a monkey that had developed yaws; one with splenic juice from the same monkey; one with serum from a yaws papilloma in a monkey; and two with serum from a primary syphilitic lesion. In addition, one monkey after recovery from yaws was reinoculated with human yaws serum, and two after recovery were inoculated with syphilis. As regards results; of the five monkeys inoculated with yaws serum taken immediately from the human lesions all developed typical yaws tubercles; the animal inoculated with serum from a yaws lesion in a monkey died before the period of incubation, as shown by our experiments, had expired; the monkey reinoculated with yaws after recovery has developed no lesions. Lastly, in not one of the four monkeys inoculated with syphilis have any lesions developed.

PERIOD OF INCUBATION.

As will be seen upon referring to the protocols, the period of incubation of yaws in the monkeys we experimented with varied from sixteen to about forty-five days, but it should be understood that this is only approximate, as owing to the distance of the location of the animals from us and pressure of work, the animals were not inspected every day and thus the lesions may have existed a short time before they were

noticed. However, the limit of error in this respect is small and of no practical importance. The approximate period of incubation in our five successful inoculations was as follows:

	Days.
Monkey No. 1 (3070)	16
Monkey No. 2 (3071)	20
Monkey No. 3 (3072)	20
Monkey No. 4 (3073)	20
Monkey No. 5 (A)	35 to 45

In the case of monkey No. 5 (A), the yaws lesion, when first noticed, was about the size of a small pea and had obviously been present for a number of days.

Comparing our results with these of Neisser, Baermann and Halberstädter(28), it is noticeable that in our monkeys the period of incubation was much shorter, as a rule, although the same low type of animal was used. Indeed, the incubation period of yaws in *Cynomolgus philippinensis* Geoff., approaches more nearly that in Gibbons, as is shown by the investigators mentioned. Thus, in the lower types of monkeys used by them, the incubation period in five animals was found to be twenty-two, thirty-one, sixty-five, ninety-one and ninety-six days respectively, while in only one of our five animals did it probably exceed twenty days. If we add to this result the probability that the lesions in all of our cases may have existed for a day or two before they were noticed, thus shortening the period of incubation still further, the difference in our results and those in the German commission becomes more noticeable. The regularity of the period in our animals is also worthy of notice, four of them developing the disease between the fifteenth and twentieth day after inoculation.

Duration of the disease.—In the five monkeys in which we produced frambœsia by inoculation the duration of the lesion was as follows:

Monkey No. 1 (3070), eighty-four days; No. 2 (3071), fifty-seven days; No. 3 (3072), ten days (this animal was chloroformed while the lesions were still active); No. 4 (3073), thirty-nine days; No. 5 (A), fourteen to twenty-one days.

It was invariably our experience that in the more severe cases the primary lesion tended to spread into the surrounding skin, and the more marked this tendency was, the longer the disease lasted. We failed to observe any general glandular enlargement or any symptoms pointing to a general infection.

The lesions of frambœsia as observed in monkeys.—The lesions produced by the experimental inoculation or frambœsia in monkeys do not differ essentially, in their morphology, from those occuring in the disease in man, but we have never observed the secondary or generalized eruption, which, according to most authors, follows the primary lesion in the human subject. Neisser, Baermann and Halberstädter regard as secondary eruptions the extension of the infection from the site of the

original lesion and in one of our animals, Monkey No. 1 (3070), such an extension occurred. However, we do not believe the new lesions so produced to constitute a secondary eruption, but simply to be an invasion of the contiguous healthy tissue by the organism causing the disease; that is, the treponema. Castellani appears to have secured true secondary lesions situated at a distance from the original papule in his one successful inoculation, and in this case a general infection might be supposed to exist.

The evidence obtained from our experiments would appear to indicate that experimental frambœsia in the monkey, at least in *Cynomolgus philippinensis* Geoff., is a purely local infection which readily heals after a period of time varying from a few days to several weeks. As we have stated, a few days after inoculation, the wounds had completely healed, although when the infection was conveyed by means of a subcutaneous pocket a slight thickening about the site of inoculation persisted for a short time, finally disappearing before the appearance of the yaws papule.

In all of our animals the yaws lesion appeared at the site of inoculation and when first diagnosed consisted of a small papule, very slightly elevated above the surrounding skin and covered with a yellowish cap or crust. The papules varied in size from that of a large pin's head to a small pea. The epidermis had been replaced by the yellowish crust, which upon removal revealed a moist surface composed of minute, closely aggregated, but separate, pinkish points, from which a thin, slightly milky fluid exuded.

The initial papule gradually enlarged, became in most instances elevated, and the crust, formed of the exuded serum, became thicker and more noticeable. The lesions were circular in form and firm upon pressure. Even when fully developed they were not greatly elevated, as is so frequently the case in human yaws tubercles, and in only one of our animals did they project markedly above the surrounding surface. While the crust covering them was always more or less elevated, it would almost invariably be found upon its removal that the granulating surface was but slightly raised, although very distinctly demarcated from the healthy skin surrounding it.

The crust covering the fully developed yaws lesion in the monkey was perfectly characteristic of that over similar lesions in man, varying in thickness, easily removed, and yellowish-brown in color, sometimes streaked with reddish-brown due to admixtures with blood.

The surface of the fully developed yaws papule in the monkey, after the removal of the crust, was typical of that observed in human lesions. The color varied from a light pink to a bright red, and a colorless or slightly whitish serum oozed from the raw surface which consisted of minute, closely aggregated papillæ, situated upon a slightly raised base and surrounded by apparently healthy skin. In some of our animals the typical "raspberry" appearance, so characteristic of the human yaws

tubercle, was well illustrated. When fully developed the papules averaged 1 centimeter in diameter. In one of our animals, Monkey No. 1 (3070), the lesion both upon the head and the abdomen was typical of that variety of the disease described by Pierez(29), Scheube(30), Manson(31) and others as "ringworm yaws." The first lesion appeared upon the abdomen and presented the appearance already described. After a few days it was observed that in both the abdominal lesion and that which had meanwhile appeared on the head, healing was occurring at the center while the edges were covered with an elevated crust. At this time the lesion resembled a ringworm infection so closely that we made an examination for the fungus, with negative results.

The lesion upon the head, when fully developed, measured about 2 centimeters in diameter and consisted of a perfect ring of raised, granulating tissue covered with the characteristic yellowish crust, and inclosing the original site of the yaws papule, which had healed without scar formation and but little pigmentation. Removal of the crust disclosed the usual moist, pink surface and an examination of the serum exuding from it demonstrated the presence of *Treponema pertenuis* in large numbers. A slight extension of this lesion occurred in the form of a small, characteristic papule developing at its lower portion and slightly involving the eyelid.

The abdominal lesion enlarged rapidly and for some time presented the appearance of a large yaws tubercle, markedly elevated and covered with a mammillated yellow crust. Healing began at the center of the tubercle and soon a typical, "ringworm" appearance was assumed but here a very considerable invasion of the surrounding skin occurred, new papules appeared at the periphery of the original lesion, so that eventually nearly one-half of the surface of the abdomen was involved in the process. The new lesions were easily demonstrated to be extensions, in direct continuity with preëxisting ones and sound skin was never found separating these lesions while in the active stage. Their progress answered perfectly to the so-called secondary lesions described by Neisser, Baermann and Halberstädter, but as we have stated, we can not regard them as an evidence of a general infection and therefore as "secondary" in the sense in which the term is used in connection with syphilis.

After persisting for a varying period of time, the lesions of frambœsia heal in the same manner as those occurring in man, the hypertrophied papillæ atrophy, the crust covering the papilloma shrivels up and falls off, and a slightly discolored, but apparently sound area of skin, devoid of hair, marks their former site. After a few days the hair again grows and it becomes practically impossible to discover the point of the inoculation. As it is now nearly three months since our animals have recovered from the infection, and as we have seen no evidence of a generalized secondary eruption, we believe we are justified in asserting that

in the species of monkey we used, a general eruption of yaws does not occur after experimental inoculation. While the lesions of frambœsia are undoubtedly modified somewhat in the monkeys of the low type used in our work, they are yet so characteristic that we believe, from their appearance alone, a clinical diagnosis could be made even in the mildest case of infection we have observed, while in the more severe infections, such as Monkey No. 1 (3070), the nature of the lesion was apparent at a glance. It is probable that if higher species of apes were used, the lesions would be much more pronounced and a generalized eruption of yaws tubercles might occur.

Examination for Treponema pertenuis.—We have examined the lesions in all of our sucessfully inoculated animals for *Treponema pertenuis* and have repeatedly demonstrated its presence in every case, without any special difficulty. The organisms occurred usually in the very earliest stage of growth of the yaws papule and persisted until the lesion had nearly healed, being most numerous during the active growth of the papule and decreasing in number as the healing process advanced. As we have stated, the treponema occurring in the lesions in monkeys did not differ in any particular from the ones found in the serum from the lesions in man. In most instances no other spirochætæ were observed in the preparation, although, in one or two cases, organisms corresponding to the type of *S. refrigens* were observed, but these were very rare. As in man, the lesions covered with crust showed the treponema unmixed with other spirochætæ, while in those in which the crust had been removed, for instance by scratching, thus allowing secondary infections to occur, organisms corresponding to the types described by Castellani were infrequently observed.

Serum from the lesions in some of our inoculated animals was collected in capillary tubes and kept for varying periods of time. Apparent multiplication of *Treponema pertenuis* occurred in some, and the organisms remained motile for several days. In the material so collected the organisms occurred singly, in pairs, or in clumps. Agglutination and apparent longitudinal division were also observed in the serum from the lesions in these animals.

We consider the constant presence of *Treponema pertenuis* in the experimental lesions of yaws in monkeys, produced by the inoculation from the lesions in man, of serum containing them and their absence in other conditions, to be conclusive proof of their etiological relationship to frambœsia. If we add to this the fact that as the lesions heal, the treponema gradually disappear and the further fact, as proved by the case of Monkey No. 2 (3071), that the organisms can not be found in pyogenic ulcerations even when inoculated, unless frambœsia be induced, it appears to us that the evidence is complete. *Treponema pertenuis* is found constantly and only in the lesions of frambœsia, whether they are

natural, as in human infection, or experimental, as in the infection of animals.

Reinfection.—In only one instance [Monkey No. 2 (3071)] have we attempted to reinfect a monkey that had recovered from frambœsia, and in this animal the reinoculation of human yaws serum resulted negatively.

Inoculation from monkey to monkey.—In one instance [Monkey No. 8 (3111)] we attempted to inoculate a monkey with the serum from a well-marked lesion occurring in another animal of the same species, but unfortunately the inoculated animal died in nineteen days, before the probable period of incubation had expired. In view of the results of Neisser, Baermann and Halberstädter, who obtained only one successful result from the inoculation of seven monkeys with the serum of infected animals, it is obvious that no conclusions can be drawn from our single experiment.

Inoculation of blood and splenic pulp.—In order to determine whether frambœsia, as observed in infected animals, is a general or local disease we inoculated one monkey [No. 6 (3109)] with blood from the heart of an animal infected with yaws, and another [No. 7 (3110)] with splenic pulp from the same animal. No results followed these inoculations, but we do not consider that the experiments prove anything as Neisser, Baermann and Halberstädter obtained only negative results in six monkeys inoculated with splenic pulp and with a mixture of splenic juice, bone marrow and mesenteric glands and only one positive result in three animals inoculated with bone marrow. We did not attempt the inoculation of bone marrow, but in view of the fact, that of the nine animals injected by the investigators mentioned, the only positive result was obtained by the inoculation of this substance, we feel that our negative result with the blood and splenic pulp does not justify us in drawing a definite conclusion as to the production of the disease in this manner.

Inoculation of yaws and syphilis.—Both Castellani(32) and Neisser and his co-workers appear to have proved conclusively that monkeys which have recovered from yaws are susceptible to syphilis. We have endeavored to repeat their experiments, but have failed to produce syphilis either in monkeys which have recovered from yaws or in those that have never suffered from the disease.

As shown in the protocols of our experiments, we inoculated two animals, Monkey No. 1 (3070) and Monkey No. 4 (3073), both of which had recovered from well-marked yaws lessions, with serum from a chancre containing at the time of inoculation numerous examples of *Treponema pallidum*. As controls we inoculated two healthy animals with serum from the same case. At the present time, two months after inoculation, none of these monkeys has developed syphilitic lesions,

and we are forced to the conclusion that it is extremely difficult, if not impossible, to inoculate syphilis in the species of monkeys used in our experiments (*Cynomolgus philippinensis* Geoff.). This difficulty, compared with the ease with which frambœsia in transmitted to the same species, speaks very strongly against the identity of the two diseases.

Yaws and syphilis.—As is well known, the question of the relation of yaws to syphilis has always excited much controversy, and Hutchinson's theory that yaws is the original form of syphilis, the latter disease, as we observe it to-day, being frambœsia modified by passage through the Caucasian race, still has many supporters. The discovery of an organism in yaws lesions indistinguishable morphologically from *Treponema pallidum,* at first sight appeared to lend additional evidence to the claim that yaws and syphilis are identical, but the experimental evidence already at hand demonstrates that the lesions produced by *Treponema pertenuis* differ greatly from those caused by *Treponema pallidum,* and that infection with one of these organisms does not produce immunity against the other. *Treponema pertenuis* and *Treponema pallidum* are, therefore, distinct species, and the lesions produced by each are characteristic and easily distinguished clinically, in uncomplicated cases.

There is no room for doubt in our minds, after consulting the work of other authors and investigators and our own clinical and experimental experience, that yaws and syphilis are distinct diseases, our belief being based upon the following facts:

(*a*) The pleomorphism of the lesions of syphilis, the uniformity of those of yaws.

(*b*) The granulomata (yaws papules) are the primary lesions of yaws; such lesions, if syphilitic, could only be *secondary* or *tertiary.*

(*c*) The presence of the very peculiar and typical yellow cap, or crust, covering the yaws lesions.

(*d*) In infected regions every uncomplicated case of yaws, whether in children or adults, presents the same characteristic lesion (the papule covered with a yellow crust.) If the disease were syphilitic a wider variation in the type of the lesion would be observed.

(*e*) The epidemic occurrence of yaws, especially among young children, and the greater prevalence of the disease in children.

(*f*) The absence of genital infections in any case observed by us.

(*g*) The absence in yaws of such striking symptoms as loss of hair and iritis.

(*h*) The auto-inoculability of yaws, even when a general eruption is present.

(*i*) The ready inoculability of yaws into such a low type of monkey as *Cynomolgus philippinensis* Geoff., and the negative result of the inoculation of syphilis in this species of monkey.

(*j*) The fact, as proved by Neisser, Baermann, and Halberstädter, and by Castellani, that monkeys susceptible to both yaws and syphilis can be infected with both, no immunity being conferred against the one by an attack of the other.[2]

(*k*) The fact, as proved by Charlouis (33) and Powell (34), that patients suffering from yaws can be infected with syphilis.

GENERAL CONCLUSIONS.

As a result of our observations, both clinical and experimental, we believe that we are justified in drawing the following conclusions:

1. That *Treponema pertenuis* is the cause of yaws.
2. That *Treponema pertenuis* is constantly present in the serum from yaws lesions.
3. That the variations in the morphology of *Treponema pertenuis* are explainable by the deformities produced during the preparation of the serum for examination.
4. That *Treponema pertenuis* and *Treponema pallidum* can be differentiated by the results obtained from the inoculation of monkeys.
5. That the inoculation of the serum from human yaws lesions containing *Treponema pertenuis* causes yaws in monkeys and that the organism can easily be demonstrated in the lesions of the infected animals.
6. That the length of the period of incubation in *Cynomolgus philippinensis* Geoff. is approximately twenty days.
7. That the duration of the inoculated disease in this species of monkey varies from twenty-one to eighty-four days.
8. That yaws and syphilis are distinct diseases.
9. That *Treponema pertenuis* can be demonstrated in sections of yaws papillomata by the Levaditi method.

Castellani, in an article published in the Journal of Hygiene for July, 1907, and only reaching here after the preceding paper had gone to the printer, draws the following summary and conclusions:

"*1.* Monkeys are susceptible to yaws. The skin eruption in the monkeys I have experimented with (*Semnopithecus priamus* and *Macacus pileatus*) is, as a rule, confined to the seat of inoculation, but the infection is general, as is

[2] On September 6, 1907, all of the experimental monkeys, except the two mentioned above as dead and monkey No. 2 (3071), which was killed, were examined and none of them showed signs of either syphilis or yaws. One of the monkeys utilized in the experiments detailed in this report [No. 2 (3071)] was killed on July 22, 1907, because of the extension of the pyogenic ulcer on his brow to the orbit. All of the others are still living on October 7, 1907, and one of them [No. 11 (D)] has given birth to young. No one of them has shown signs or symptoms of either yaws or syphilis since they were last noted in the report.

proved by the presence of the *Spirochæta pertenuis* in the spleen and lymphatic glands.

"*2.* Material obtained from persons suffering from yaws and apparently containing *Spirochæta pertenuis* only is infective to monkeys.

"*3.* When the *Spirochæta pertenuis* has been removed from this material by filtration, the latter becomes inert.

"*4.* The inoculation of blood from the general circulation and blood taken from the spleen of yaws patients into monkeys may give positive results.

"*5.* The inoculation of the cerebro-spinal fluid of yaws patients gives negative results.

"*6.* Monkeys successfully inoculated with yaws do not become immune for syphilis.

"*7.* Monkeys successfully inoculated with syphilis do not become immune for yaws.

"*8.* By means of the Bordet-Gengou reaction it is possible to detect specific yaws antibodies and antigen.

"*9.* Yaws antibodies and antigen are entirely different from syphilitic antibodies and antigen.

"*10.* The presence of the *Spirochæta pertenuis* in monkeys experimentally inoculated, as well as in yaws patients, is practically constant in the unbroken eruptive lesions; the *Spirochæta* is frequently present in the spleen and lymphatic glands.

"*11.* Yaws is generally conveyed by actual contact, but under certain circumstances it may be conveyed by flies and possibly by other insects."

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ILLUSTRATIONS.

PLATE I.

FIG. 1. *T. pertenuis* from human yaws lesion. 1 × 1500 (approximate).
2. *T. pertenuis* from human yaws lesion. 1 × 1500 (approximate).
3. *T. pertenuis* from human yaws lesion. 1 × 1500 (approximate).
4. *T. pertenuis* from human yaws lesion. 1 × 1200 (approximate).
5. *T. pertenuis* from capillary tube culture, showing agglutination and probable longitudinal division. 1 × 1200 (approximate). Note comparative size of treponema and cocci.
6. Same. 1 × 1500.

PLATE II.

FIG. 7. *T. pertenuis* from capillary tube culture, showing agglutination and probable longitudinal division. 1 × 1500 (approximate). Note comparative size of treponema and cocci.
8. *T. pertenuis* from inoculated yaws in monkey. 1 × 300 (approximate).
9. *T. pertenuis* from inoculated yaws in monkey. 1 × 1500 (approximate).
10. *T. pallidum* from human syphilis. 1 × 1500 (approximate).
11. *T. pertenuis* in degenerated area of epithelium of human yaws lesion. Levaditi method.

PLATE III.

FIGS. 12–15. Examples of yaws lesions as seen in Filipinos.

PLATE IV.

Diagrammatic illustration of types exhibited by *T. pertenuis* in stained preparations.

FIG. 1.

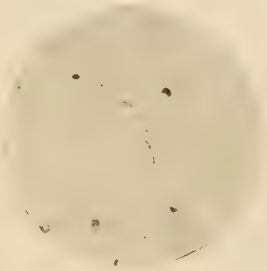


FIG. 2.

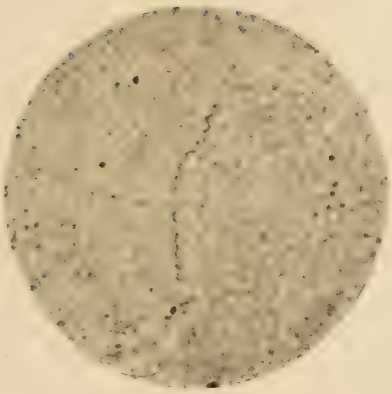
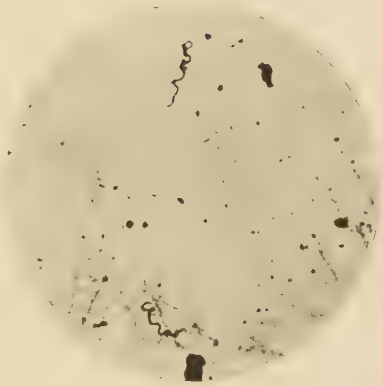


FIG. 3.



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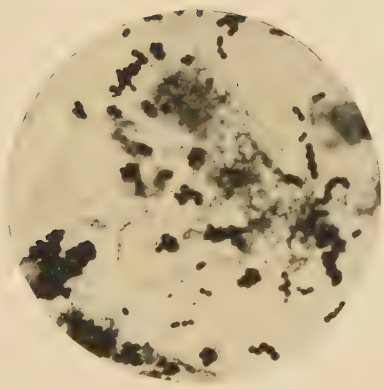

FIG. 5.

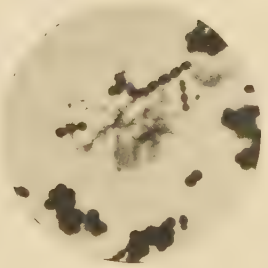

FIG. 6.

PLATE I.

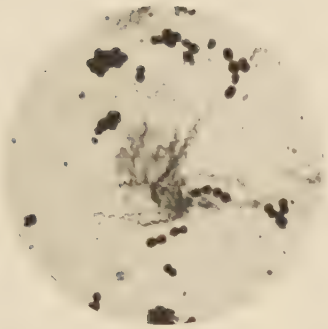

Fig. 7.

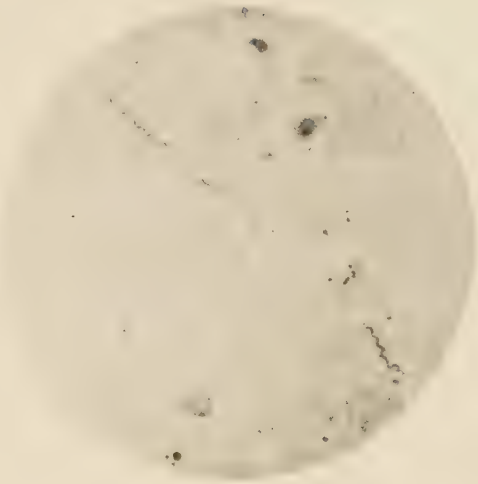

Fig. 8.

Fig. 9.

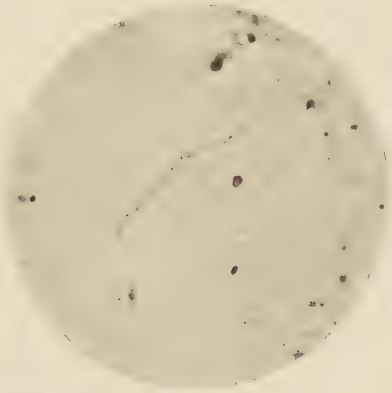

Fig. 10.

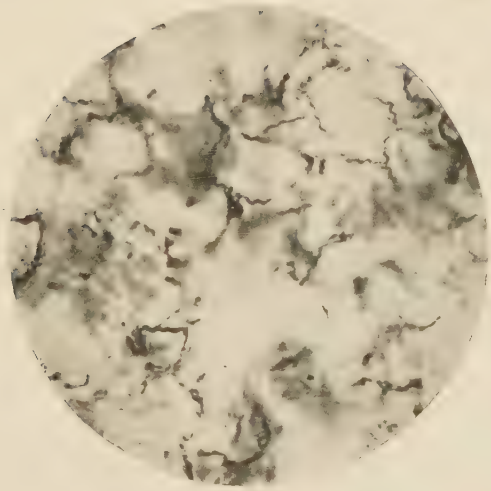

Fig. 11.

PLATE II.

FIG. 12.

FIG. 13.

FIG. 14.

FIG. 15.

PLATE III.

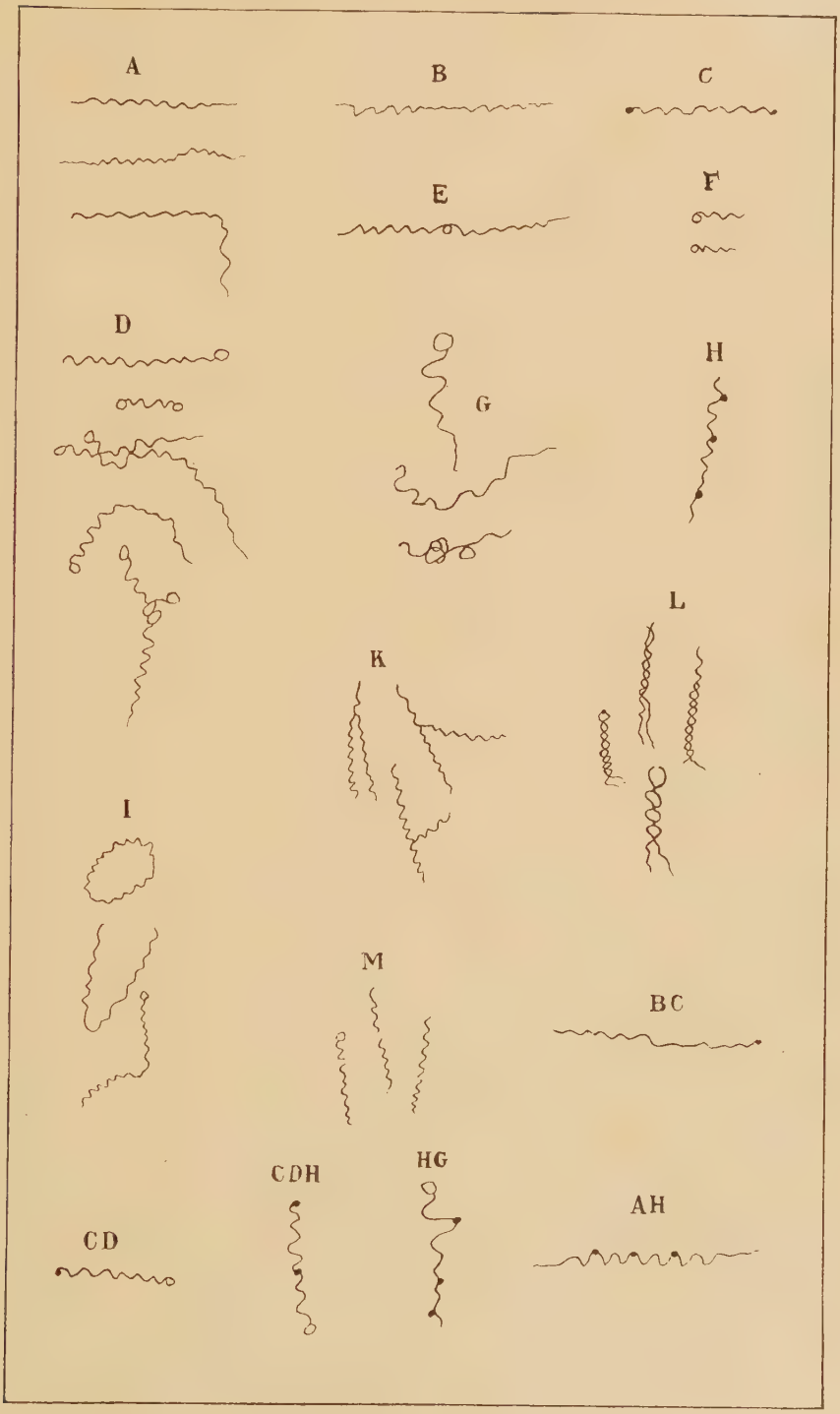

PLATE IV.

YAWS: A HISTOLOGIC STUDY.

By Harry T. Marshall.
(*From the Biological Laboratory, Bureau of Science.*)

Doctors Ashburn and Craig kindly turned over to me the tissues from the cases discussed in the preceding paper, for study. The tissues examined consisted (1) of an early yaws papule removed from a patient suffering with leprosy and yaws, and (2) of ulcerating yaws nodules from a monkey inoculated from a patient seen near Manila.

As the photographs illustrate, the lesions in the two cases are essentially alike and a single description suffices for them.

Sections through the papule and ulcers were fixed in Zenker's fluid immediately after removal and were treated with the ordinary stains. Hæmatoxylin and eosin gave the clearest pictures. The sections show (*a*) regressive, (*b*) vascular and exudative, and (*c*) regenerative changes. The regressive ones are confined to the epithelial structures; the exudative are most marked immediately beneath the epithelium; the regenerative changes are evidenced as atypical epithelial formations and by the presence of a few epithelioid connective tissue cells.

(*a*) The degenerative changes are confined to the epithelial structures, including surface epithelium, epithelial downgrowths, hair follicles, sebaceous and sweat glands. There is at first an increase in the thickness of the epithelium affecting chiefly the polyhedral cells, with blurring of the outlines between the separate layers and disappearance of the pigment layer. The horny layer is thrown off and the epithelium appears as a greatly thickened structure, made up of large, swollen, cloudy, vacuolated cells, with swollen, pale nuclei, showing irregular dots of chromatin. The columnar cell layer is the only one that is well preserved. Where the disease is more advanced, the surface layers have been thrown off, exposing either the columnar layer of cells or the subjacent corium. The ulcer is coated in places with a crust of necrotic material, leucocytes, etc. (See Pl. I, fig. 1, *a, b, c, e.*) Similar changes appear in the epithelial downgrowths, the hair follicles, and to a lesser extent in the sweat glands; the centrally placed epithelial cells being swollen, very cloudy, vacuolated, and often replaced by cavities containing a few leucocytes and a small amount of granular débris, while the peripheral one or two rows of cells preserve fairly well their columnar shape, alignment,

and staining properties. (See Pls. I and II, figs. 1 *c*; 2 *b*; 3.) Cross sections through an epithelial downgrowth or hair follicle with central softening, may be slightly suggestive of the "pearls" of epithelioma. (See Pls. I and III, figs. 1 *e*; 2 *c*; 4.) Charlouis, Unna, and others note especially that the hair follicles and hair shafts are unaffected, but this does not hold true of the nodules now under consideration. The sections from the human case did not pass through any hair follicles, but in the sections from monkeys, hair follicles are abundant and the outer root sheath shows exactly the same necrotic changes as are met with in the other epithelial structures. The sweat glands and sebaceous glands are altered less than the hair follicles and downgrowths.

(*b*) The vascular and exudative changes are marked in the corium, and extend only a short distance into the heavier strands of subcutaneous tissue. There is a very great dilatation of the capillaries extending to the under surface of epithelium. Many capillaries are empty, others contain polymorphonuclears, eosinophiles and a few mononuclears, while others are full of erythrocytes. At first sight it appears as if most of the erythrocytes are free in the tissue, but in my sections I have been able to make out a limiting capillary wall in most cases and find only small extravasations. The corium is markedly œdematous, the œdema extending into the epithelium and for a slight distance into the subcutaneous tissue. The early yaws papule contains many leucocytes and the ulcerating nodule is packed with them. A few polymorphonuclears are found in the epithelial structures, while the downgrowths and other degenerating parts are surrounded with collections of polymorphonuclears, large and small mononuclears, many of which are plasma cells, with occasional eosinophiles. A few lymphocytes are found and only a small number of extravasated erythrocytes. The eosinophiles have polymorphous nuclei. The leucocytes are also found both within capillaries and surrounding them, and it is most probable that they have arrived by way of the blood vessels and have wandered out from them. The distribution of eosinophiles is interesting. At the edge of the nodule, beyond the line of epithelial degeneration and at a point where the œdema and leucocytic infiltration is not very great, the number of eosinophiles is both relatively and absolutely greater than it is beneath the center of the lesion where the infiltration is denser. The eosinophiles vary from 9 to 35 in one field of the microscope (Zeiss DD objective, No. 3 eyepiece). They are scattered diffusely, but occur in greater numbers around and in the dilated capillaries. (For exudative changes see Pls. I and II, figs. 1, 2, 3.) At no point is there any evidence of the perivascular infiltration with mononuclears, which is so characteristic of syphilis.

(*c*) Regenerative changes. There is indication of slight new formation of capillaries in the corium and of a minor degree of connective tissue

new growth, but the most obvious changes occur in the epithelium. At the edges of the ulcer there is a marked increase in the thickness of epithelium and a striking increase in the number and size of the epithelial downgrowths. Irregularities and distortions in the epithelial downgrowths are also seen beneath the surface of the ulcer, from which the epithelial covering has been lost. (See Pl. I, figs. 1 *d;* 2.) It is evident that, while new growth of epithelium is occurring from the columnar layer, degenerative changes are taking place in the older, more centrally placed portions of the epithelial downgrowths. (See Pls. I, II, and III, figs. 1 *e;* 3 *a;* 4.)

Tissues from these cases were treated by the method of Levaditi, with negative results, but through the courtesy of Drs. Ashburn and Craig I have had an opportunity to examine a successful silver stain of a yaws papule prepared in Washington from tissue obtained in the Philippines. In this section enormous numbers of spirochætæ are found in the degenerating central parts of the epithelial downgrowths. The spirochætæ are free in the necrotic material resulting from breaking down of epithelial cells. None were found within cells, within nuclei, nor in any part of the corium.

SUMMARY OF FINDINGS.

We may conclude from a study of these specimens, that we are dealing with a primary degenerative change resembling colliquative necrosis, affecting the epithelial structures and caused by spirochætæ, which are very abundant in the necrotic material, at least at some stage of the disease. The degeneration leads to ulcer formation. Following the degeneration there is irregular, new formation of epithelium in the form of downgrowths, which in turn often degenerate. Accompanying these changes vascular dilatation, œdema and leucocytic infiltration occur in the corium, with a minor degree of new formation of capillaries and connective tissue. There is no endarteritis, nor are there any other changes suggestive of syphilis. The majority of the infiltrating cells are polymorphonuclears at an early stage of the lesion, while mononuclears, many of which are of the plasma cell type, are almost equally abundant. In the tissue from the human being the plasma cells outnumber the polymorphonuclears. Polymorphonuclear eosinophiles are abundant and have a peculiar distribution.

The changes are essentially the same in lesions from monkeys and human beings.

The histological characteristics of the yaws nodules have been studied by Charlouis, Unna, Glogner, Plehn and others.

Charlouis (*Vierteljahresschrift (Archiv) f. Dermatologie und Syphilis* (1881), 431, quoted by Unna and others) describes particularly the epithelial overgrowth and the leucocytic infiltration, the hair follicles escaping, while the

sebaceous glands are enlarged. Glogner (*Virchow's Archiv.* (1902), **168**, 443) notes in addition a reduction in the connective and elastic tissue fibrils in the large nodules, and the occurrence of occasional pigment cells and free erythrocytes in the tissues. He did not find plasma cells but found mast cells and a few giant cells. He also encountered a lymphocytosis in the blood of 30 to 50 per cent of the patients. Plehn (Mense, Handbuch der Tropenkrankheiten (1906), **2**, 60) states that the hair follicles and sweat glands are unaffected, that the cutis is infiltrated with cells, chiefly plasma cells, and that the nodule presents the histologic picture of a granuloma, which can be distinguished from syphilis (1) by the fact that in yaws the primary affection is in the epithelium, in syphilis it is in the cutis, (2) in yaws the œdema is greater and there is no periarteritis nor endarteritis, (3) the infiltrating cells are of a different character in yaws, and there are no signs of necrosis in this disease. He found no giant cells.

The most detailed description is that given by Unna. (*Die Histopathologie der Hautkrankheiten.* Orth's Pathologische Anatomie, Berlin (1894), Ergänzungsband **2**, 503.) He quotes Pontoppidan (*Yaws und Framboesia* (1882), 201) as stating that the primary seat of the affection is in the prickle cell layer of the epithelium. Unna's description is based upon the study of a large, full-grown dry yaws nodule. The nodule rose abruptly from the level of the skin. The epithelial overgrowth began at the border and rapidly increased in extent within the limits of the nodule. The papillæ were not more numerous, but were ten or twenty times as long as usual. There was marked hyperkeratosis with accumulations of a many-layered, horny covering. Acanthosis led here, as in condyloma, to the formation of prickle cell masses, principally as interpapillary projections, while the superficial layer of prickle cells was reduced, and occasional, minute hæmorrhages escaped through into the crust above. Epithelial mitoses were infrequent. At the periphery, the granular layer was reduced, with crust formation from fibrin and leucocytes, at other places the granular layer was increased in thickness. Fibrin and leucocytes were found also between the deeper epithelial cells. The chief change in the cutis was a solid, cellular infiltration consisting chiefly of beautiful, large plasma cells. These surrounded like a mantle the epithelial projections and spread in thin lines into the separate papillæ. Beneath the epithelial growths they spread out into a uniform layer with processes extending laterally and downwards. The processes accompanied particularly the greatly dilated veins and were not directly related to the epithelial structures. The lumina of the sweat glands were dilated, and the epithelial lining swollen, while the hair follicles and hair shafts were unaltered either in the deeper tissue, where the plasma cell accumulation occurred, or while penetrating the acanthomatous part at the surface. At no place was there evidence of plasma cell degeneration, the tumor representing the purest type of plasmoma tissue.

The spindle cells of the cutis were enlarged, but not appreciably increased in numbers. Except for the usual rarefaction around the plasmoma foci, there was no progressive nor regressive change in the interstitial collaginous and elastic tissue. There was no appreciable increase in mast cells.

The pigment, which was heaped up in the basal prickle cells at the periphery of the nodule, spread in streams between the cells of the hypertrophied epithelial portion. The streams surrounded nuclei and entire, unaltered epithelial cells, producing appearances like pigment cells. There were other "pigment cells" of the same structure with two or three nuclei according to the number of epithelial cells which were surrounded by the pigment stream.

"All in all the structure of the frombœsial nodule is simple; a marked epithelial growth with hyperkeratosis accompanied by plasmoma formation in the cutis.

The absence of any degenerative changes in the plasmoma, either as giant cell formation or as fusion, makes the structure simpler than that of a syphilide of which the yaws nodule is otherwise suggestive." It is especially like a condyloma, from which it is distinguished by the greater dryness of the cutis in yaws and by the more marked keratinization. This explains the firmness and resistance of the nodule. Its cranberry form results from the overgrown papillary bodies which are covered by such a thin layer of prickle cells—that is, it is the result of the great vascular dilatation in the papillæ.

He thinks Charlouis was mistaken in interpreting his infiltrating cells as leucocytes, and suggests that they must have been plasma cells, while the leucocytes entered as a result of secondary infections. He thinks that "there is no question that the cause of frambœsia should be sought only in the first stages of the exanthem and should be looked for in the cutis." He observed the "abscess-like areas" in the keratinizing epithelium, but attributed them to secondary pyogenic infections. As another point against Charlouis' interpretation of the leucocytes he mentions that he could not find them around the dilated capillaries or veins.

Comparing these accounts, is seems clear that the histologic picture of the yaws papule at an early stage is somewhat different from that of the older nodules. The description given in this article and those given by Charlouis, Glogner, and to a less extent by Plehn, evidently refer to the younger nodules, while Unna's description is true only of the older ones.

The characteristic features of the early stage are (1) the epithelial degeneration, with (2) epithelial downgrowths into the cutis in the form of irregular columns; it seems clear that this appearance is due to the actual downgrowth of epithelium and not entirely to the upgrowth from the cutis, (3) capillary dilatation with engorgement, marked œdema and cellular infiltration limited quite sharply to the cutis and most marked at the under border of epithelium. The cells occurring are chiefly polymorphonuclears, large and small mononuclears, and plasma cells and œsinophiles. It is clear that the infiltrating cells are derived, at least in great measure, from the vessels. In addition Plehn found mast cells and Glogner giant cells. This last finding has been verified by no other writer. The changes in the fixed tissue cells of the cutis are relatively slight.

In the older nodule the chief difference concerns the infiltrating cells. The epithelial changes are the same, the œdema has largely disappeared, and the plasma-cells are present in such enormous numbers as to dominate the picture. Here again, the changes in the fixed tissue cells of the cutis are of minor extent.

Remembering the remarkable regenerative power of epithelium, and noting how slight are the degenerative and regenerative changes in the cutis, we can understand how it comes about that when recovery occurs there is so little scar formation at the seat of a yaws ulcer.

A comparison of the descriptions of the different writers mentioned above, with Unna's description of the syphilitic condyloma does not

leave a wide margin of difference. The differences are that the degeneration in yaws is confined to the epithelium, the spirochætæ being found in the degenerating areas, while the changes in the cutis are unimportant; that there is no periarterial or endarterial change in yaws; that the infiltrating cells at the early stage of the nodule are different from those in syphilis; and that there are no areas of necrosis and no giant cells in yaws, with the exception of the cases described by Glogner. The clinical appearance of yaws is so characteristic that it is surprising to find how closely the histological description agrees with that of the syphilide. It will be important to make a comparative study of the yaws papule and condyloma, examining lesions of the same ages, and using the silver impregnation method in the demonstration of the parasites. The claim is made that syphilis attacks primarily the cutis, while yaws, as we have seen above, is essentially a disease of the epithelium.[1]

[1] In an article which has recently arrived, Schüffner (*München. med. Wchnsch.* (1907), **54**, 1364) reviews one hundred and twenty-nine cases of yaws seen in Sumatra, and for the sake of completeness his article is abstracted. In one hundred and four cases he found the treponema, and of those cases examined more than once he found it in 98 per cent. The Romanowsky stain, or some modification, proved most satisfactory, especially when preceded by osmic acid or formalin vapor fixation. By the use of Levaditi's silver stain he found that the parasites occurred only in the diseased portion of the epidermis, especially in the deeper layers of prickle cells, and in this situation they were often extremely abundant. They were entirely absent elsewhere, notably in the perithelial situation common in syphilis.

While syphilis and yaws are closely parallel, he is convinced that yaws is an independent affection.

He gives a brief review of the histologic appearance, but of particular interest is his careful description of unusual skin manifestations in yaws. Under this heading he describes ring-shaped or kidney-shaped efflorescences and others which are impetiginous or vesicular. In others there was a definite roseola. In more than one-fourth of his cases there was a peculiar, macular eruption in which rounded spots from 1 to 3 centimeters in diameter were surrounded by minute papules, often becoming vesicular. Of especial interest is his description of the bone and joint pains in yaws, which he found to occur in 20 per cent of cases in adults. Periostitis was also very common. He thinks from his studies of yaws that as the result of further investigation and discrimination "syphilis will be dissolved into a group of independent diseases."

ILLUSTRATIONS.

PLATE I.

FIG. 1. Young papule from patient with leprosy and yaws. Magnification, 95 × 1. *a*, Edge of crust over the ulcer, composed of necrotic epithelium, leucocytes, etc. *b*, Hypertrophied epithelium. *c*, Epithelial downgrowth, with leucocytic infiltration. *d*, Base of ulcer, showing preservation of deepest layer of epithelium, beginning epithelial downgrowth, and covering of epithelial débris. *e*, Downgrowth, showing preservation of the columnar cell layer with degeneration of the central portion. *f*, *f*, Capillary dilatation in the œdematous cutis.

2. Early papule from monkey. Magnification, 95 × 1. *a*, Beginning loss of surface epithelium. *b*, *b*, Epithelial overgrowth. *c*, Cross section of epithelial downgrowth, showing central degeneration. *d*, *d*, *d*, Capillary dilatation in œdematous corium. *e*, Portion enlarged in Pl. II, fig. 3.

PLATE II.

FIG. 3. Portion of fig. 2 enlarged. Magnification, 415 × 1. *a*, Beginning degeneration of epithelium. *b*, *b*, *b*, Capillary dilatation.

PLATE III.

FIG. 4. Cross section through an epithelial downgrowth from an older nodule from a monkey, showing the necrosis of the central cells with preservation of younger cells at the margins. The œdema of the cutis also is well indicated. Magnification, 520 × 1.

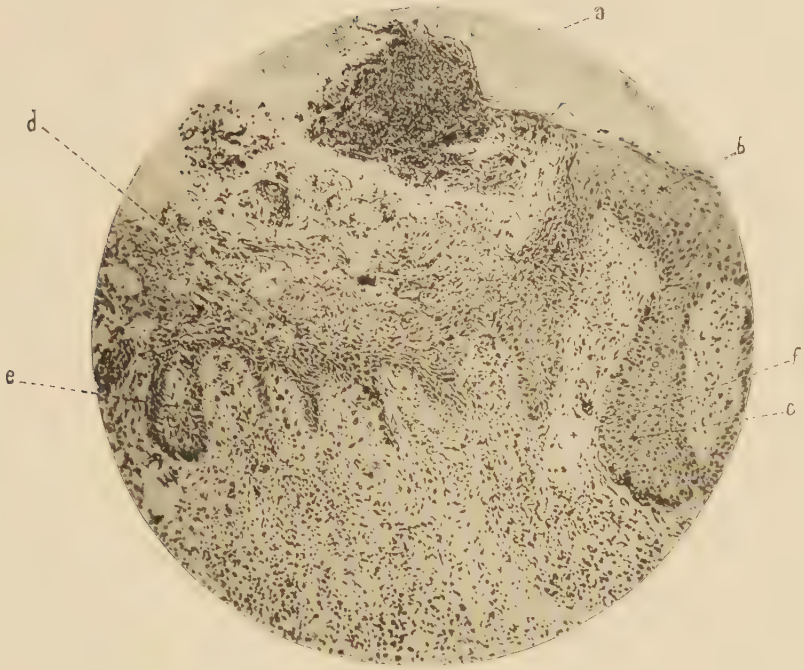

FIG. 1.

FIG. 2.

PLATE I.

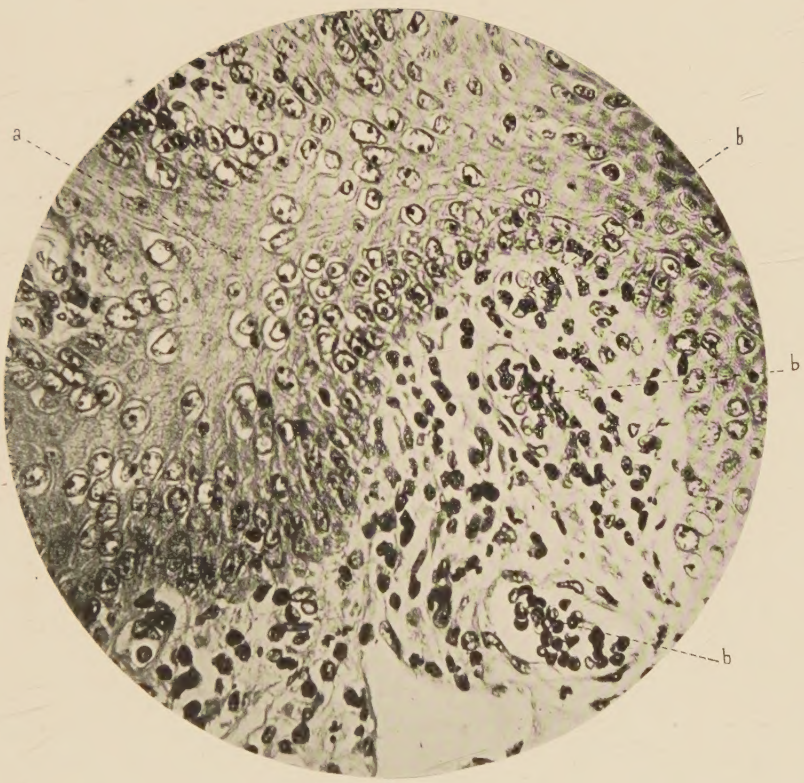

FIG. 3.

PLATE II.

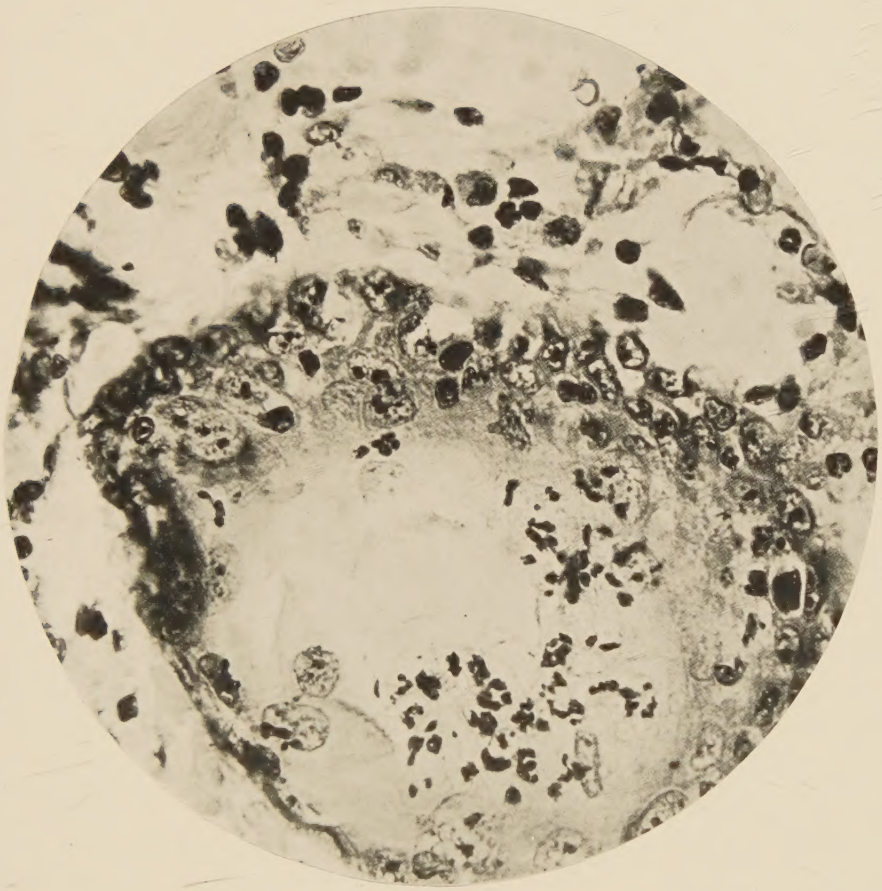

FIG. 4.

PLATE III.